

This Page Is Inserted by IFW Operations
and is not a part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

**As rescanning documents *will not* correct images,
please do not report the images to the
Image Problem Mailbox.**

(19)



Europäisches Patentamt

European Patent Office

Office européen des brevets



(11)

EP 0 425 235 B1

(12)

EUROPEAN PATENT SPECIFICATION

(45) Date of publication and mention
of the grant of the patent:
25.09.1996 Bulletin 1996/39

(51) Int. Cl.⁶: **A61K 47/48**, A61K 31/535

(21) Application number: 90311590.5

(22) Date of filing: 23.10.1990

(54) Cytotoxic agents comprising maytansinoids and their therapeutic use

Maytansinoid enthaltende cytotoxische Mittel und ihre therapeutische Anwendung

Agents cytotoxiques contenant des maytansinoides et leur application thérapeutique

(84) Designated Contracting States:
AT BE CH DE DK ES FR GB GR IT LI LU NL SE

(30) Priority: 25.10.1989 US 426247

(43) Date of publication of application:
02.05.1991 Bulletin 1991/18

(73) Proprietor: IMMUNOGEN INC
Cambridge, Massachusetts 02139 (US)

(72) Inventors:
• Chari, Ravi J.
Boston, MA 02135 (US)
• Goldmacher, Victor S.
Newton Center, MA 02159 (US)
• Lambert, John M.
Cambridge, MA 02139 (US)

• Blattler, Walter A.
Brookline, MA 02146 (US)

(74) Representative: Barnard, Eric Edward
BROOKES & MARTIN
High Holborn House
52/54 High Holborn
London WC1V 6SE (GB)

(56) References cited:
EP-A- 0 011 276 EP-A- 0 014 402
EP-A- 0 376 176

• CHEM. PHARM. BULL., vol. 32, no. 9, 1984;
A. KAWAI et al., pp. 3441-3451 ; A. KAWAI et al.
• JOURNAL OF MEDICINAL CHEMISTRY, vol. 21,
no. 1, 1978, pages 31-37, American Chemical
Society; S.M. KUPCHAN et al.

Note: Within nine months from the publication of the mention of the grant of the European patent, any person may give notice to the European Patent Office of opposition to the European patent granted. Notice of opposition shall be filed in a written reasoned statement. It shall not be deemed to have been filed until the opposition fee has been paid. (Art. 99(1) European Patent Convention).

EP 0 425 235 B1

Description

FIELD OF THE INVENTION

5 The present invention relates to novel cytotoxic agents and their therapeutic use. More specifically, the invention relates to novel cytotoxic agents comprising maytansinoids and their therapeutic use. These novel cytotoxic agents have therapeutic use as a result of delivering the maytansinoids to a specific cell population in a targeted fashion by chemically linking the maytansinoid to a cell binding agent.

10 BACKGROUND OF THE INVENTION

In recent years, a myriad of reports have appeared on the attempted specific targeting of tumor cells with monoclonal antibody-drug conjugates (Sela et al, in *Immunoconjugates* 189-216 (C. Vogel, ed. 1987); Ghose et al, in *Targeted Drugs* 1-22 (E. Goldberg, ed. 1983); Diener et al, in *Antibody mediated delivery systems* 1-23 (J. Rodwell, ed. 1988); Pietersz et al, in *Antibody mediated delivery systems* 25-53 (J. Rodwell, ed. 1988); Bumol et al, in *Antibody mediated delivery systems* 55-79 (J. Rodwell, ed. 1988). Cytotoxic drugs such as methotrexate, daunorubicin, doxorubicin, vincristine, vinblastine, melphalan, mitomycin C, and chlorambucil have been conjugated to a variety of murine monoclonal antibodies. In some cases, the drug molecules were linked to the antibody molecules through an intermediary carrier molecule such as serum albumin (Garnett et al, 46 *Cancer Res.* 2407-2412 (1986); Ohkawa et al 23 *Cancer Immunol. Immunother.* 81-86 (1986); Endo et al, 47 *Cancer Res.* 1076-1080 (1980)), dextran (Hurwitz et al, 2 *Appl. Biochem.* 25-35 (1980); Manabi et al, 34 *Biochem. Pharmacol.* 289-291 (1985); Dillman et al, 46 *Cancer Res.* 4886-4891 (1986); Shoval et al, 85 *Proc. Natl. Acad. Sci.* 8276-8280 (1988)), or polyglutamic acid (Tsukada et al, 73 *J. Natl. Canc. Inst.* 721-729 (1984); Kato et al 27 *J. Med. Chem.* 1602-1607 (1984); Tsukada et al, 52 *Br. J. Cancer* 111-116 (1985)).

25 A wide array of linker technologies have been employed for the preparation of such immunoconjugates and both cleavable and non-cleavable linkers have been investigated. In most cases, the full cytotoxic potential of the drugs could only be observed, however, if the drug molecules could be released from the conjugates in unmodified form at the target site.

One of the cleavable linkers that has been employed for the preparation of antibody-drug conjugates is an acid-labile linker based on *cis*-aconitic acid that takes advantage of the acidic environment of different intracellular compartments such as the endosomes encountered during receptor mediated endocytosis and the lysosomes. Shen and Ryser introduced this method for the preparation of conjugates of daunorubicin with macromolecular carriers (102 *Biochem. Biophys. Res. Commun.* 1048-1054 (1981)). Yang and Reisfeld used the same technique to conjugate daunorubicin to an anti-melanoma antibody (80 *J. Natl. Canc. Inst.* 1154-1159 (1988)). Recently, Dillman et al also used an acid-labile linker in a similar fashion to prepare conjugates of daunorubicin with an anti-T cell antibody (48 *Cancer Res.* 6097-6102 (1988)).

An alternative approach, explored by Trouet et al, involved linking daunorubicin to an antibody via a peptide spacer arm (79 *Proc. Natl. Acad. Sci.* 626-629 (1982)). This was done under the premise that free drug could be released from such a conjugate by the action of lysosomal peptidases.

40 *In vitro* cytotoxicity tests, however, have revealed that antibody-drug conjugates rarely achieved the same cytotoxic potency as the free unconjugated drugs. This suggested that mechanisms by which drug molecules are released from the antibodies are very inefficient. In the area of immunotoxins, conjugates formed via disulfide bridges between monoclonal antibodies and catalytically active protein toxins were shown to be more cytotoxic than conjugates containing other linkers. See, Lambert et al, 260 *J. Biol. Chem.* 12035-12041 (1985); Lambert et al, in *Immunotoxins* 175-209 (A. Frankel, ed. 1988); Ghetie et al 48 *Cancer Res.* 2610-2617 (1988). This was attributed to the high intracellular concentration of glutathione contributing to the efficient cleavage of the disulfide bond between an antibody molecule and a toxin. Despite this, there are only a few reported examples of the use of disulfide bridges for the preparation of conjugates between drugs and macromolecules. Shen et al described the conversion of methotrexate into a mercaptoethylamide derivative followed by conjugation with poly-D-lysine via a disulfide bond (260 *J. Biol. Chem.* 10905-10908 (1985)). A recent report described the preparation of a conjugate of the trisulfide-containing toxic drug calicheamycin with an antibody (Menendez et al, *Fourth International Conference on Monoclonal Antibody Immunoconjugates for Cancer, San Diego*, Abstract 81 (1989)).

One reason for the lack of disulfide linked antibody-drug conjugates is the unavailability of cytotoxic drugs possessing a sulfur atom containing moiety that can be readily used to link the drug to an antibody via a disulfide bridge. Furthermore, chemical modification of existing drugs is difficult without diminishing their cytotoxic potential.

Another major drawback with existing antibody-drug conjugates is their inability to deliver a sufficient concentration of drug to the target site because of the limited number of targeted antigens and the relatively moderate cytotoxicity of cancerostatic drugs like methotrexate, daunorubicin and vincristine. In order to achieve significant cytotoxicity, linkage of large number of drug molecules either directly to the antibody or through a polymeric carrier molecule becomes nec-

essary. However such heavily modified antibodies often display impaired binding to the target antigen and fast *in vivo* clearance from the blood stream.

Maytansinoids are highly cytotoxic drugs. Maytansine was first isolated by Kupchan et al from the east African shrub *Maytenus serrata* and shown to be 100 to 1000 fold more cytotoxic than conventional cancer chemotherapeutic agents like methotrexate, daunorubicin, and vincristine (U.S. Patent No. 3,896,111). Subsequently it was discovered that some microbes also produce maytansinoids, such as maytansinol and C-3 esters of maytansinol (U.S. Patent No. 4,151,042). Synthetic C-3 esters of maytansinol and analogues of maytansinol have also been reported (Kupchan et al, 21 *J. Med. Chem.* 31-37 (1978); Higashide et al, 270 *Nature* 721-722 (1977); Kawai et al, 32 *Chem. Pharm. Bull.* 3441-3451 (1984)). Examples of analogues of maytansinol from which C-3 esters have been prepared include maytansinol with modifications on the aromatic ring (e.g. dechloro) or at the C-9, C-14 (e.g. hydroxylated methyl group), C-15, C-18, C-20 and C-4,5.

The naturally occurring and synthetic C-3 esters can be classified into two groups:

(a) C-3 esters with simple carboxylic acids (U.S. Patent Nos. 4,248,870; 4,265,814; 4,308,268; 4,308,269; 4,309,428; 4,317,821; 4,322,348; and 4,331,598), and

(b) C-3 esters with derivatives of *N*-methyl-L-alanine (U.S. Patent Nos. 4,137,230; 4,260,608; and 12 *Chem. Pharm. Bull.* 3441 (1984)).

Esters of group (b) were found to be much more cytotoxic than esters of group (a).

Maytansine is a mitotic inhibitor. Treatment of L1210 cells *in vitro* with maytansine has been reported to result in 67% of the cells accumulating in mitosis. Untreated control cells were reported to demonstrate a mitotic index ranging from between 3.2 to 5.8% (Sieber et al, 43 *Comparative Leukemia Research 1975, Bibl. Haemat.* 495-500 (1976)). Experiments with sea urchin eggs and clam eggs have suggested that maytansine inhibits mitosis by interfering with the formation of microtubules through the inhibition of the polymerization of the microtubule protein, tubulin (Remillard et al, 189 *Science* 1002-1005 (1975)).

In vitro P388, L1210, and LY5178 murine leukemic cell suspensions have been found to be inhibited by maytansine at doses of 10^{-3} to 10^{-7} microgram/ml, with the P388 line being the most sensitive. Maytansine has been also shown to be an active inhibitor of *in vitro* growth of human nasopharyngeal carcinoma cells and the human acute lymphoblastic leukemia line C.E.M. was reported inhibited by concentrations as low as 10^{-7} microgram/ml (Wolpert-DeFillippes et al, 24 *Biochem. Pharmacol.* 173501738 (1975)).

In vivo, maytansine has also been shown to be active. Tumor growth in the P388 lymphocytic leukemia system was shown to be inhibited over a 50- to 100-fold dosage range which suggested a high therapeutic index; also significant inhibitory activity could be demonstrated with the L1210 mouse leukemia system, the human Lewis lung carcinoma system and the human B-16 melano-carcinoma system (Kupchan, 33 *Ped. Proc* 2288-2295 (1974)).

Because the maytansinoids are highly cytotoxic, they were expected to be of use in the treatment of many diseases such as cancer. This expectation has yet to be realized. Clinical trials with maytansine were not favourable due to a number of side effects (Issel et al, 5 *Can. Trtmnt. Rev.* 199-207 (1978)). Adverse effects to the central nervous system and gastrointestinal symptoms were responsible for some patients refusing further therapy (Issel at 204), and it appeared that maytansine was associated with peripheral neuropathy that might be cumulative (Issel at 207).

Accordingly, compositions for treating diseases with maytansinoids where their side effects are reduced without compromising their cytotoxicity is greatly needed.

EP-276176 claims priority dates of 27th December 1988 and 26th January 1989 and was published on 4th July, 1990 EP-376176 describes ansamitocin derivatives. Ansamitocins (ANS) belong to the general class of compounds called maytansinoids since they have the same basic ring structure. They differ from the subgroup containing maytansine because they lack an *N*-methyl-L-alanine substituent. The ansamitocin derivatives described in EP-376176 are not chemically linked to a cell binding agent.

SUMMARY OF THE INVENTION

Thus, one object of the present invention is to provide maytansinoids in a form that are highly cytotoxic and that can still be effectively used in the treatment of many diseases.

Another object of the present invention is to provide novel maytansinoid esters.

These and other objects have been achieved by providing a cytotoxic agent comprising one or more maytansinoids, chemically linked to a cell binding agent.

In a second embodiment, the present invention provides a therapeutic agent for killing selected cell populations comprising:

- (a) a cytotoxic amount of one or more maytansinoids linked to a cell binding agent, and
- (b) a pharmaceutically acceptable carrier, diluent or excipient.

In a third embodiment, the present invention provides a method other than a method for treatment of the human or animal body by surgery or therapy or diagnostic methods practiced on the human or animal body for killing selected cell populations comprising contacting a cell population or tissue suspected of containing cells from said selected cell population with a cytotoxic amount of a cytotoxic agent comprising one or more maytansinoids linked to a cell binding agent.

In a fourth embodiment, the present invention provides an *N*-methyl-alanine-containing ester of maytansinol or an analogue of maytansinol, said *N*-methyl-alanine-containing ester comprising a linking group capable of linking an *N*-methyl-alanine-containing maytansinoid ester to a chemical moiety.

In a fifth embodiment, the present invention provides an *N*-methyl-cysteine-containing ester of maytansinol or an analogue of maytansinol.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows maytansine (1a) and maytansinol (1b).

Fig. 2 shows the synthesis of disulfide-containing derivatives of *N*-methyl-*L*-alanine.

Fig. 3 shows the synthesis of disulfide- and thiol-containing maytansinoids which can be linked to cell binding agents via a disulfide or any other sulfur-containing link such as thioether or thioester links. The synthesis starts with the intermediates of Fig. 2.

Fig 4(A) shows the synthesis of disulfide- and thiol-containing derivatives of *N*-methyl-*L*-cysteine.

Fig. 4(B) shows the synthesis of disulfide- and thiol-containing maytansinoids from the intermediates of Fig 4(A) that can be conjugated to cell binding agents via a disulfide or any other sulfur-containing link such as thioether or thioester links.

Fig. 5 shows graphically the cytotoxicity of antibody-maytansinoid conjugates.

Figs. 6(A) and 6(B) show graphically results of competitive binding assays of antibody-maytansinoid conjugates.

Fig. 7 shows graphically the blood clearance of an antibody-maytansinoid conjugate in mice.

DETAILED DESCRIPTION OF THE INVENTION

This invention is based on the synthesis of novel maytansinoid derivatives that retain high cytotoxicity and that can be effectively linked to cell binding agents. The art reveals that it is extremely difficult to modify existing drugs without diminishing their cytotoxic potential. The disclosed invention overcomes this problem by modifying the disclosed maytansinoid derivatives with chemical moieties, especially ones containing thiol or disulfide groups, to which appropriate cell binding agents can be linked. As a result, the disclosed novel maytansinoid derivatives preserve and in some cases even enhance the cytotoxic potency of the naturally occurring maytansinoids. The cell binding agent-maytansinoid derivative conjugates permit the full measure of the cytotoxic action of the maytansinoid derivatives to be applied in a targeted fashion against unwanted cells only, therefore, avoiding side effects due to damage to non-targeted healthy cells. This invention permits the maytansinoid derivatives to live up to their potential, something their undirected cytotoxic effects has previously made impossible. Thus the invention provides useful agents for the elimination of diseased or abnormal cells that are to be killed or lysed such as tumor cells (particularly solid tumor cells), virus infected cells, microorganism infected cells, parasite infected cells, autoimmune cells (cells that produce autoantibodies), activated cells (those involved in graft rejection or graft vs. host disease), or any other type of diseased or abnormal cells, while exhibiting a minimum of side effects.

Thus, this invention teaches the synthesis of maytansinoid derivatives that can be chemically linked to a cell binding agent while keeping a high cytotoxicity either in bound form or in released form or in both states. High cytotoxicity is defined as exhibiting a toxicity having an IC_{50} - the inhibiting concentration of a toxic substance that leaves a surviving fraction of 0.5 - of about 10^{-8} M or less when measured *in vitro* with KB cells upon a 24 hour exposure time to the drug.

Cytotoxic Agent

The cytotoxic agent according to the present invention comprises one or more maytansinoids linked to a cell binding agent.

In order to link the maytansinoid to a cell binding agent, the maytansinoid must first be modified.

Maytansinoids that can be used in the present invention to produce the modified maytansinoid capable of being linked to a cell binding agent are well known in the art and can be isolated from natural sources according to known method or prepared synthetically according to known methods.

Examples of suitable maytansinoids include maytansinol and maytansinol analogues. Examples of suitable maytansinol analogues include those having a modified aromatic ring and those having modifications at other positions.

Specific examples of suitable analogues of maytansinol having a modified aromatic ring include:

- (1) C-19-dechloro (U.S. Patent #4,256,746) (prepared by LAH reduction of ansamycin P2);
 (2) C-20-hydroxy (or C-20-demethyl) +/-C-19-dechloro (U.S. Patent #4,361,650 and #4,307,016) (prepared by demethylation using *Streptomyces* or *Actinomyces* or dechlorination using LAH); and
 (3) C-20-demethoxy, C-20-acyloxy (-OCOR), +/-dechloro (U.S. Patent #4,294,757) (prepared by acylation using acyl chlorides).

Specific examples of suitable analogues of maytansinol having modifications of other positions include:

- (1) C-9-SH (U.S. Patent #4,424,219) (prepared by the reaction of maytansinol with H₂S or P₂S₅);
 (2) C-14-alkoxymethyl(demethoxy/CH₂OR) (U.S. Patent #4,331,598);
 (3) C-14-hydroxymethyl or acyloxymethyl (CH₂OH or CH₂OAc) (U.S. Patent #4,450,254) (prepared from *Nocardia*);
 (4) C-15-hydroxy/acyloxy (U.S. Patent #4,364,866) (prepared by the conversion of maytansinol by *Streptomyces*);
 (5) C-15-methoxy (U.S. Patent #4,313,946 and #4,315,929) (isolated from *Trewia nudiflora*);
 (6) C-18-N-demethyl (U.S. Patent #4,362,663 and #4,322,348) (prepared by the demethylation of maytansinol by *Streptomyces*); and
 (7) 4,5-deoxy (U.S. Patent #4,371,533) (prepared by the titanium trichloride/LAH reduction of maytansinol).

In order to link chemically the maytansinoid to the cell binding agent, a linking group is used.

Suitable linking groups are well known in the art and include disulfide groups, thioether groups, acid labile groups, photolabile groups, peptidase labile groups and esterase labile groups. Preferred are disulfide groups and thioether groups.

According to the present invention the linking group is part of a chemical moiety that is covalently bound to the maytansinoid through conventional methods. In a preferred embodiment, the chemical moiety can be covalently bound to the maytansinoid via an ester linkage.

Many positions on maytansinoids are expected to be useful as the linkage position, depending upon the type of link. For example, for forming an ester linkage, the C-3 position having a hydroxyl group, the C-14 position modified with hydroxymethyl, the C-15 position modified with hydroxy and the C-20 position having a hydroxy group are all expected to be useful. However the C-3 position is preferred and the C-3 position of maytansinol is especially preferred.

Also preferred is an *N*-methyl-alanine-containing C-3 ester and an *N*-methyl-cysteine-containing C-3 ester of maytansinol or its analogues.

Synthesis of Esters of Maytansinol Having a Linking Group

While the synthesis of esters of maytansinol having a linking group is described below in terms of thiol and disulfide linking groups, one of skill in the art will understand that other linking groups, specific representative examples of which are set forth in Example 4, can also be used with the present invention as can other maytansinoids.

The synthesis of maytansinoid derivatives can be described by reference to Figures 1, 2, 3, 4(A) and 4(B), where disulfide-containing maytansinoid esters are prepared by condensing maytansinol **1b** with freshly prepared *N*-methyl-*L*-alanine or *N*-methyl-*L*-cysteine derivatives containing a disulfide group.

ω -Mercapto-carboxylic acids of varying chain lengths are converted into their respective methyl-dithio, e.g. **3a** to **3d** (where *n* = 1-10, including branched and cyclic aliphatics), or aryl-dithio, e.g. **4a** to **4b**, derivatives by reacting them with methyl methanethiosulfonate or aryl disulfides, such as diphenyldisulfide and ring substituted diphenyldisulfides and heterocyclic disulfides such as 2,2'-dithiopyridine. The carboxylic acids are activated and then reacted with *N*-methyl-*L*-alanine to form the desired carboxylic acid compounds, e.g. **5a** to **5f** for condensation with maytansinol **1b**.

Esterification of maytansinol **1b** or an analogue with the carboxylic acids **5a** to **5f** gives the disulfide-containing maytansinoids **6a** to **6f**. Cleavage of the disulfide group in **6a** to **6f** with dithiothreitol gives the thiol-containing maytansinoids **7a** to **7c**, which readily linked via disulfide or thioether links to cell binding agents.

N-methyl-*L*-alanine can be prepared as described in the literature (See, Fu, S.J. & Birnbaum, S.M., 75 *J. Amer. Chem. Soc.* 918 (1953); or is obtainable commercially (Sigma Chemical Company)).

In another embodiment, *N*-methyl-cysteine or *N*-methyl-homocysteine can be converted to the respective disulfide derivatives **8** (*n*=1 and 2, respectively), which are then acylated to yield the desired carboxylic acids **9** (*n*=1 and 2, respectively). Maytansinol is then esterified with **9** (*n* = 1) to give disulfide-containing ester **10**. Reduction of **10a** with dithiothreitol as described for **7b** produces the thiol-containing maytansinoid **11** which can be conjugated to cell binding agents.

N-methyl-cysteine can be prepared as described in Undheim and Eidem, 23 *Acta Chem. Scand.* 3129-3133 (1970).

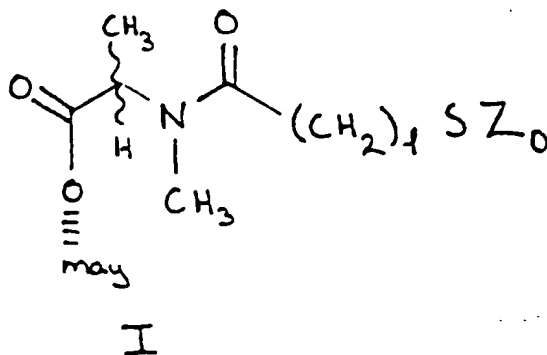
More specifically, maytansinol **1b** is derived from maytansine **1a** or other esters of maytansinol by reduction such as with lithium aluminum hydride. (Kupchan, S.M. et al 21 *J. Med. Chem.* 31-37 (1978); U.S. Patent #4,360,462). It is also possible to isolate maytansinol from the microorganism *Nocardia*, see, Higashide et al, U.S. Patent #4,151,042

(1979). Maytansinol is then converted to the different ester derivatives, 6a to 6f and 10, using a suitable agent such as dicyclohexylcarbodiimide (DCC) and catalytic amounts of zinc chloride (See, U.S. Patent No. 4,137,230; Kawai et al, 32 *Chem. Pharm. Bull.* 3441-3951 (1984); U.S. Patent No. 4,260,609). The two diastereomeric products containing the *D* and *L*-aminoacyl side chains result. The diastereomeric maytansinoid esters are readily separated by preparative
 5 TLC on silica gel. For example, using Analtech GF plates (1000 micrometers) and developing with 6% methanol in chloroform yields distinct banding; the desired bands are scraped off the plate and the products extracted with ethyl acetate. See, Kupchan, S.M., 21 *J. Med. Chem.* 31-37 (1978); and Higashide et al, U.S. Patent No. 4,360,462 (1982).

Reduction of the disulfide-containing maytansinoids to the corresponding mercapto-maytansinoids 7a, 7b, 7c and 11, is achieved by treatment with dithiothreitol (DTT) and purification by HPLC using a Waters radialpak C-18 column
 10 and eluting with a linear gradient of 55% to 80% acetonitrile in H₂O over 10 min. at a flow rate of 1.5 ml/min.

When analogues of maytansinol are used as the starting material to give analogous disulfide-containing maytansinoid esters, the analogues are prepared before reacting them with the *N*-methyl-*L*-alanine or *N*-methyl-*L*-cysteine derivatives.

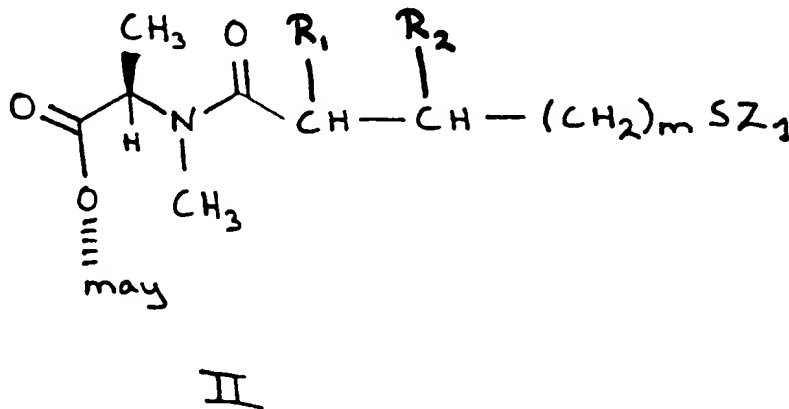
Specific examples of *N*-methyl-alanine-containing maytansinoid derivatives useful in the present invention are represented by the formulae (I), (II), (III) and (IV).
 15



wherein:

Z_0 represents H or SR, wherein R represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted
 35 aryl or heterocyclic;

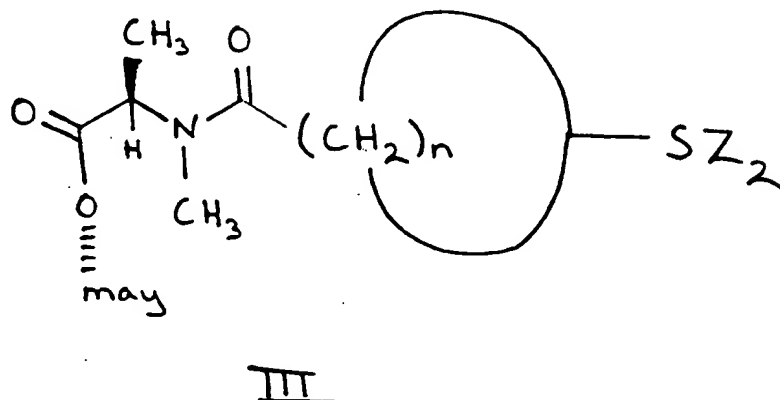
l represents an integer of 1 to 10; and
 may represents a maytansinoid.



wherein:

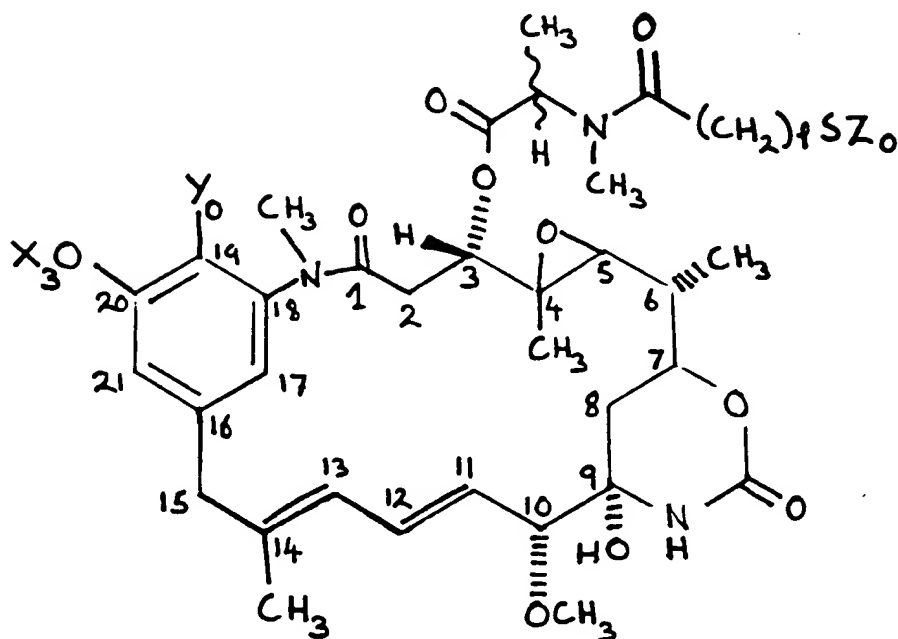
R_1 and R_2 , which may be the same or different, represents H, CH₃ or CH₂CH₃;

Z_1 represents H or SR_3 , wherein R_3 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;
 m represents 0, 1, 2 or 3; and
 may represents a maytansinoid.



wherein:

Z_2 represents H or SR_4 , wherein R_4 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;
 n represents an integer of 3 to 8; and
 may represents a maytansinoid.



IV

wherein:

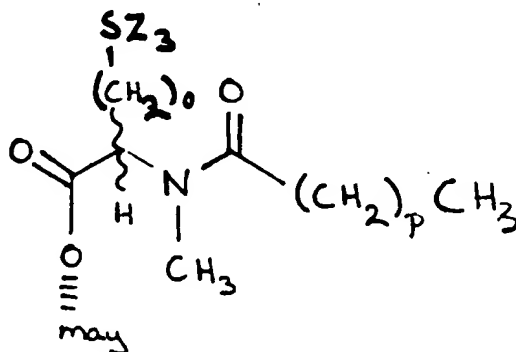
Z_0 represents H or SR, wherein R represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic;

l represents 1, 2 or 3;

y_0 represents Cl or H; and

X_3 represents H or CH_3 .

Specific examples of *N*-methyl-cysteine-containing maytansinoid derivatives useful in the present invention are represented by the formulae (V) and (VI).



V

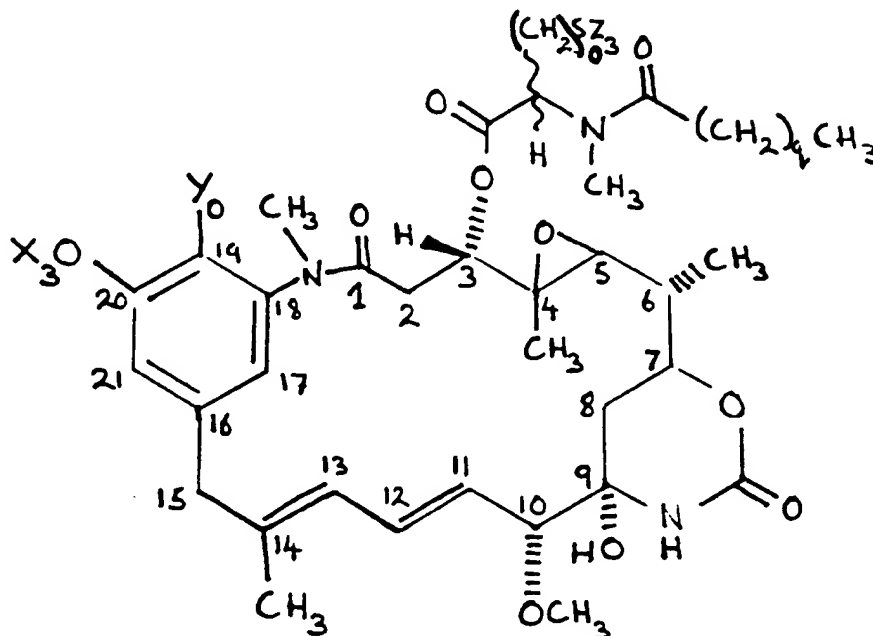
wherein:

Z_3 represents H or SR_5 , wherein R_5 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;

o represents 1, 2 or 3;

p represents 0 or an integer of 1 to 10; and

may represents a maytansinoid.



VI

wherein:

Z_3 represents H or SR_5 , wherein R_5 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic;

o represents 1, 2, or 3;

q represents 0 or an integer of 1 to 10;

Y_0 represents Cl or H; and

X_3 represents H or CH_3 .

Examples of linear alkyls include methyl, ethyl, propyl, butyl, pentyl and hexyl.

Examples of branched alkyls include isopropyl, isobutyl, sec.-butyl, tert.-butyl, isopentyl and 1-ethyl-propyl.

Examples of cyclic alkyls include cyclopropyl, cyclobutyl, cyclopentyl and cyclohexyl.

Examples of simple aryls include phenyl and naphthyl.

Examples of substituted aryls include aryls such as those described above substituted with alkyl groups, with halogens, such as Cl, Br, F, nitro groups, amino groups, sulfonic acid groups, carboxylic acid groups, hydroxy groups and alkoxy groups.

Examples of heterocyclics are compounds wherein the heteroatoms are selected from O, N and S, and include pyrrol, pyridyl, furyl and thiophene.

Disulfide-containing and mercapto-containing maytansinoid drugs of the invention can be evaluated for their ability to suppress proliferation of various unwanted cell lines *in vitro*. For example, cell lines such as the human epidermoid carcinoma line KB, the human breast tumor line SKBR3, and the Burkitt's lymphoma line Namalwa can easily be used for the assessment of cytotoxicity of these compounds. Cells to be evaluated can be exposed to the compounds for 24 hours and the surviving fractions of cells measured in direct assays by known methods. IC_{50} values can then be calculated from the results of the assays.

Preparation of Cell Binding Agents

The effectiveness of the compounds of the invention as therapeutic agents depends on the careful selection of an appropriate cell binding agent. Cell binding agents may be of any kind presently known, or that become known and include peptides and non-peptides. Generally, these can be antibodies (especially monoclonal antibodies), lymphokines, hormones, growth factors, nutrient-transport molecules (such as transferrin), or any other cell binding molecule or substance.

More specific examples of cell binding agents that can be used include:

- monoclonal antibodies;
- fragments of antibodies such as Fab, Fab', and F(ab')₂ (Parham, 131 *J. Immunol.* 2895-2902 (1983); Spring et al, 113 *J. Immunol.* 470-478 (1974); Nisonoff et al, 89 *Arch. Biochem. Biophys.* 230-244 (1960));
- interferons (e.g. α , β , γ);
- lymphokines such as IL-2, IL-3, IL-4, IL-6;
- hormones such as insulin, TRH (thyrotropin releasing hormone), MSH (melanocyte-stimulating hormone), steroid hormones, such as androgens and estrogens;
- growth factors and colony-stimulating factors such as EGF, TGF- α , G-CSF, M-CSF and GM-CSF (Burgess, 5 *Immunology Today* 155-158 (1984)); and
- transferrin (O'Keefe et al, 260 *J. Biol. Chem.* 932-937 (1985)).

Monoclonal antibody techniques allow for the production of extremely specific cell binding agents in the form of specific monoclonal antibodies. Particularly well known in the art are techniques for creating monoclonal antibodies produced by immunizing mice, rats, hamsters or any other mammal with the antigen of interest such as the intact target cell, antigens isolated from the target cell, whole virus, attenuated whole virus, and viral proteins such as viral coat proteins. Sensitized human cells can also be used.

Selection of the appropriate cell binding agent is a matter of choice that depends upon the particular cell population that is to be targeted, but in general monoclonal antibodies are preferred if an appropriate one is available.

For example, the monoclonal antibody J5 is a murine IgG_{2a} antibody that is specific for the Common Acute Lymphoblastic Leukemia Antigen (CALLA) (Ritz et al, 283 *Nature* 583-585 (1980)) and can be used if the target cells express CALLA such as in the disease of acute lymphoblastic leukemia. Similarly, the monoclonal antibody anti-B4 is a murine IgG₁, that binds to the CD19 antigen on B cells (Nadler et al, 131 *J. Immunol.* 244-250 (1983)) and can be used if the target cells are B cells or diseased cells that express this antigen such as in non-Hodgkin's lymphoma or chronic lymphoblastic leukemia.

Additionally, GM-CSF which binds to myeloid cells can be used as a cell binding agent to diseased cells from acute myelogenous leukemia. IL-2 which binds to activated T-cells can be used for prevention of transplant graft rejection, for therapy and prevention of graft-versus-host disease, and for treatment of acute T-cell leukemia. MSH which binds to melanocytes can be used for the treatment of melanoma.

Cancers of the breast and testes can be successfully targeted with estrogen (or estrogen analogues) or androgen (or androgen analogues) respectively as cell binding agents.

Preparation of Cytotoxic Agents or Conjugates

Conjugates of the maytansinoid derivatives of the invention and a cell binding agent can be formed using any techniques presently known or later developed. The maytansinoid ester can be modified to yield a free amino group and then linked to an antibody or other cell binding agent via an acid-labile linker, or a photolabile linker. The maytansinoid ester can be condensed with a peptide and subsequently linked to a cell binding agent to produce a peptidase-labile linker. The maytansinoid ester can be treated to yield a primary hydroxyl group, which can be succinylated and linked to a cell binding agent to produce a conjugate that can be cleaved by intracellular esterases to liberate free drug. Most preferably, the maytansinoid esters are treated to create a free or protected thiol group, and then one or many disulfide or thiol-containing maytansinoid derivatives are covalently linked to the cell binding agent via disulfide bond(s).

Representational conjugates of the invention are antibody/maytansinoid derivatives, antibody fragment/maytansinoid derivatives, epidermal growth factor (EGF)/maytansinoid derivatives, melanocyte stimulating hormone (MSH)/maytansinoid derivatives, thyroid stimulating hormone (TSH)/maytansinoid derivatives, estrogen/maytansinoid derivatives, estrogen analogue/maytansinoid derivatives, androgen/maytansinoid derivatives, androgen analogue/maytansinoid derivatives.

Maytansinoid conjugates of antibodies, antibody fragments, protein hormones, protein growth factors and other proteins are made in the same way. For example, peptides and antibodies can be modified with crosslinking reagents such as *N*-succinimidyl 3-(2-pyridyldithio)propionate, 4-succinimidyl-oxycarbonyl- α -methyl- α -(2-pyridyldithio)-toluene (SMPT), *N*-succinimidyl-3-(2-pyridyldithio)-butyrate (SDPB), 2-iminothiolane, or *S*-acetylsuccinic anhydride by known

methods. See, Carlsson et al, 173 *Biochem. J.* 723-737 (1978); Blattler et al, 24 *Biochem.* 1517-1524 (1985); Lambert et al, 22 *Biochem.* 3913-3920 (1983); Klotz et al, 96 *Arch. Biochem. Biophys.* 605 (1962); and Liu et al, 18 *Biochem.* 690 (1979), Blakey and Thorpe, 1 *Antibody, Immunoconjugates & Radiopharmaceuticals*, 1-16 (1988), Worrell et al 1 *Anti-Cancer Drug Design* 179-184 (1986). The cell binding agent containing free or protected thiol groups thus derived is then reacted with a disulfide- or thiol-containing maytansinoid to produce conjugates. The conjugates can be purified by HPLC or by gel filtration.

Similarly, for example, estrogen and androgen cell binding agents such as estradiol and androstenediol can be esterified at the C-17 hydroxy group with an appropriate disulfide-containing carboxylic acid using e.g. dicyclohexylcarbodiimide as a condensing agent. Examples of such carboxylic acids that can be employed are 3-(2-pyridyldithio)propanoic acid, 3-methyldithiopropionic acid, and 3-phenyldithiopropionic acid. Esterification of the C-17 hydroxy group can also be achieved by reaction with an appropriately protected thiol group-containing carboxylic acid chloride such as 3-S-acetylpropanoyl chloride. Other methods of esterification can also be employed as described in the literature (Haslam, 36 *Tetrahedron* 2409-2433 (1980)). The protected or free thiol-containing androgen or estrogen can then be reacted with a disulfide or thiol-containing maytansinoid to produce conjugates. The conjugates can be purified by column chromatography on silica gel or by HPLC.

Preferably monoclonal antibody or cell binding agent/maytansinoid conjugates are those that are joined via a disulfide bond, as discussed above, that are capable of delivering maytansinoid molecules. Such cell binding conjugates are prepared by known methods such as modifying monoclonal antibodies with succinimidyl pyridyl-dithiopropionate (SPDP) (Carlsson et al, 173 *Biochem. J.* 723-737 (1978)). The resulting thiopyridyl group is then displaced by treatment with thiol-containing maytansinoids to produce disulfide linked conjugates. Alternatively, in the case of the arylthiomaytansinoids, the formation of the cell binding conjugate is effected by direct displacement of the aryl-thiol of the maytansinoid by sulfhydryl groups previously introduced into antibody molecules. Conjugates containing 1 to 10 maytansinoid drugs linked via a disulfide bridge are readily prepared by either method.

More specifically, a solution of the dithiopyridyl modified antibody at a concentration of 1 mg/ml in 0.1 M potassium phosphate buffer, at pH 7.0 containing 1 mM EDTA is treated with the thiol-containing maytansinoid (1.25 molar equivalent/dithiopyridyl group). The release of pyridine-2-thione from the modified antibody is monitored spectrophotometrically at 343nm and is complete in about 30 min. The antibody-maytansinoid conjugate is purified and freed of unreacted drug and other low molecular weight material by gel filtration through a column of Sephadex® G-25. The number of maytansinoids bound per antibody molecule can be determined by measuring the ratio of the absorbance at 252nm and 280nm. An average of 1-10 maytansinoid molecules/antibody molecule can be linked via disulfide bonds by this method.

Antibody-maytansinoid conjugates with non-cleavable links can also be prepared. The antibody can be modified with crosslinking reagents such as succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS), sulfo-MBS or succinimidyl-iodoacetate, as described in the literature, to introduce 1-10 reactive groups. See, Yoshitake et al, 101 *Eur. J. Biochem.* 395-399 (1979); Hashida et al, *J. Applied Biochem.* 56-63 (1984); and Liu et al, 18 *Biochem.* 690-697 (1979). The modified antibody is then reacted with the thiol-containing maytansinoid derivative to produce a conjugate. The conjugate can be purified by gel filtration through a Sephadex® G-25 column.

The modified antibodies are treated with the thiol-containing maytansinoid (1.25 molar equivalent/maleimido group). The mixtures are incubated overnight at about 4°C. The antibody-maytansinoid conjugates are purified by gel filtration through a Sephadex® G-25 column. Typically, an average of 1-10 maytansinoids/antibody are linked.

A preferred method is to modify antibodies with succinimidyl 4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (SMCC) to introduce maleimido groups followed by reaction of the modified antibody with a thiol-containing maytansinoid to give a thioether-linked conjugate. Again conjugates with 1 to 10 drug molecules per antibody molecule result.

Cytotoxicity Assays

Cytotoxicity of the maytansinoids and their antibody conjugates to non-adherent cell lines such as Namalwa and HL-60 can be measured by back-extrapolation of cell proliferation curves as described in Goldmacher et al, 135 *J. Immunol.* 3648-3651 (1985). Cytotoxicity of these compounds to adherent cell lines such as SKBR3 and KB can be determined by clonogenic assays as described in Goldmacher et al, 102 *J. Cell Biol.* 1312-1319 (1986).

Therapeutic Agent and Method for Killing Selected Cell Populations.

The present invention also provides a therapeutic agent for killing selected cell populations comprising:

- (a) a cytotoxic amount of one or more maytansinoids linked to a cell binding agent, and
- (b) a pharmaceutically acceptable carrier, diluent or excipient.

Similarly, the present invention provides a method other than a method for treatment of the human or animal body by surgery or therapy or diagnostic methods practiced on the human or animal body for killing selected cell populations comprising contacting a cell population or tissue suspected of containing cells from said selected cell population with a cytotoxic amount of a cytotoxic agent comprising one or more maytansinoids linked to a cell binding agent.

The cytotoxic agent is prepared as described above.

Suitable pharmaceutically acceptable carriers, diluents, and excipients are well known and can be determined by those of skill in the art as the clinical situation warrants.

Examples of suitable carriers, diluents and/or excipients include: (1) Dulbecco's phosphate buffered saline, pH about 7.4, containing about 1 mg/ml to 25 mg/ml human serum albumin, (2) 0.9% saline (0.9% w/v NaCl), and (3) 5% (w/v) dextrose.

The method for killing selected cell populations can be practiced *in vitro*, *in vivo*, or *ex vivo*.

Examples of *in vitro* uses include treatments of autologous bone marrow prior to their transplant into the same patient in order to kill diseased or malignant cells: treatments of bone marrow prior to their transplantation in order to kill competent T cells and prevent graft-versus-host-disease (GVHD); treatments of cell cultures in order to kill all cells except for desired variants that do not express the target antigen; or to kill variants that express undesired antigen.

The conditions of non-clinical *in vitro* use are readily determined by the skilled artisan.

For clinical *ex vivo* use to remove tumor cells or lymphoid cells from bone marrow prior to autologous transplantation in cancer treatment or in treatment of autoimmune disease, or to remove T cells and other lymphoid cells from autologous or allogeneic bone marrow or tissue prior to transplantation in order to prevent GVHD, treatment can be carried out as follows. Bone marrow is harvested from the patient or other individual and then incubated in medium containing serum to which is added the cytotoxic agent of the invention, concentrations range from about 10 μ M to 1 pM, for about 30 minutes to about 48 hours at about 37°C. The exact conditions of concentration and time of incubation (= dose) are readily determined by the skilled artisan. After incubation the bone marrow cells are washed with medium containing serum and returned to the patient by i.v. infusion according to known methods. In circumstances where the patient receives other treatment such as a course of ablative chemotherapy or total-body irradiation between the time of harvest of the marrow and reinfusion of the treated cells, the treated marrow cells are stored frozen in liquid nitrogen using standard medical equipment.

For clinical *in vivo* use, the cytotoxic agent of the invention will be supplied as solutions that are tested for sterility and for endotoxin levels. Examples of suitable protocols of conjugate administration are as follows. Conjugates are given daily for 5 days either as an i.v. bolus each day for 5 days, or as a continuous infusion for 5 days. Bolus doses are given in 50 to 100 ml of normal saline to which 5 to 10 ml of human serum albumin has been added. Continuous infusions are given in 250 to 500 ml of normal saline, to which 25 to 50 ml of human serum albumin has been added, per 24 hour period. Dosages will be 10 μ g to 100 mg/kg of body weight per day, i.v. (range of 1 ng to 10 mg/kg per day). Four weeks after treatment, the patient may receive a second course of treatment. Specific clinical protocols with regard to route of administration, excipients, diluents, dosages, times, etc., can be determined by the skilled artisan as the clinical situation warrants.

Examples of medical conditions that can be treated according to the *in vivo* or *ex vivo* methods of killing selected cell populations include malignancy of any type including, for example, cancer of the lung, breast, colon, prostate, kidney, pancreas, ovary, and lymphatic organs; autoimmune diseases, such as systemic lupus, rheumatoid arthritis, and multiple sclerosis; graft rejections, such as renal transplant rejection, liver transplant rejection, lung transplant rejection, cardiac transplant rejection, and bone marrow transplant rejection; graft versus host disease; viral infections, such as CMV infection, HIV infection, AIDS, etc.; and parasite infections, such as giardiasis, amoebiasis, schistosomiasis, and others as determined by one skilled in the art.

EXAMPLES

The invention will now be illustrated by reference to non-limiting examples. Unless otherwise stated, all percents, ratios, parts, etc. are by weight.

EXAMPLE 1

Synthesis of Maytansinoid Derivatives

Melting points were determined on a Electrothermal melting point apparatus. Proton magnetic resonance (1 H NMR) spectra were obtained on a Varian EM360 spectrometer at 60 MHz or on a Bruker AM300 machine at 300 MHz. Chemical shifts are reported in δ values relative to an internal tetramethylsilane (TMS) standard. UV spectra were recorded on a Perkin Elmer λ 4A spectrophotometer. Optical rotations were determined using a Perkin Elmer model 241 polarimeter. A Rainin HPX instrument equipped with a Gilson variable wavelength UV detector and a Waters Radialpak C-18 column was used for HPLC analyses. Elemental analyses were performed by Atlantic Microlabs, Atlanta, Ga.

3-Methyldithio-propanoic acid (3b). To a stirred solution of 3-mercaptopropanoic acid (**2b**) (5.00 g, 0.047 mol) in water (150 ml), cooled in an ice bath, was added methyl methanethiosulfonate (6.54 g, 0.052 mol) in absolute ethanol (75 ml). The reaction mixture was stirred overnight at room temperature. The mixture was then diluted with saturated, aqueous NaCl (400 ml) and extracted with ether (3 x 150 ml). The combined ether extracts were washed with saturated NaCl, dried over Na₂SO₄ and concentrated. The residue was distilled to afford a colorless liquid (6.47 g, 90%): bp_{1.0} 105°C. ¹H NMR(CDCl₃) δ 2.3 (3H, s), 2.8 (4H, m), 11.2 (1H, s).

4-methyldithio-butanoic acid (3c). To a stirred solution of bis-(3-carboxypropyl)-disulfide (1.00 g, 4.20 mmol) in methanol (20 ml) was added a solution of dithiothreitol (0.647 g, 4.20 mmol) in H₂O (20 ml). A solution of 10 M NaOH (0.842 ml, 8.42 mmol) was then added and the mixture was allowed to stir at room temperature overnight to effect complete reduction. Methyl methanethiosulfonate (1.17 g, 9.24 mmol) was added and the reaction mixture allowed to stir for another three hours. The mixture was then diluted with saturated, aqueous NaCl (150 ml), acidified (aqueous HCl), and extracted with ethyl ether (3 x 100 ml). The combined organic layers were washed with saturated NaCl, dried (Na₂SO₄), concentrated and the concentrate was chromatographed on silica gel eluting with methylene chloride/ethyl acetate to give 0.867 g (56%) of a clear liquid. ¹H NMR (CDCl₃) δ 2.1 (2H, m), 2.4 (3H, s), 2.4 (2H, m), 2.7 (2H, m), 11.1 (1H, s).

5-Mercaptopentanoic acid (2d). Compound **2d** was prepared by a modification of a literature method (Khim et al, 37 *J. Org. Chem.* 2714-2720 (1972)). To a stirred solution of 5-bromopentanoic acid (1.80 g, 0.01 mol) in absolute ethanol (25 ml) was added thiourea (0.761 g, 0.01 mol) and the reaction mixture was refluxed for 6 hours. A solution of 50% aqueous NaOH (20 ml) was then added and the mixture refluxed for an additional two hours. The mixture was then diluted with water (100 ml), acidified (aqueous HCl), and extracted with ethyl acetate (4 x 50 ml). The combined organic layers were dried over sodium sulfate and evaporated under reduced pressure. The residue was chromatographed over silica gel eluting with methylene chloride/ethyl acetate to give 0.885 g (66%) of a colorless liquid. ¹H NMR (CDCl₃) δ 1.3 (1H, t), 1.6 (4H, m), 2.4 (4H, m), 11.5 (1H, s).

5-Methyldithio-pentanoic acid (3d). To a stirred solution of 5-mercaptopentanoic acid (**2d**) (0.500 g, 3.73 mmol) in water (20 ml) was added a solution of methyl methanethiosulfonate (0.517 g, 4.10 mmol) in absolute ethanol (5 ml) and the mixture was stirred at room temperature for 3 hours. The mixture was then diluted with aqueous, saturated NaCl (100 ml) and extracted with ethyl ether (3 x 100 ml). The combined organic layers were washed with saturated NaCl, dried (Na₂SO₄), evaporated under reduced pressure and the concentrate was chromatographed over silica gel eluting with methylene chloride/ethyl acetate to yield 0.602 g (90%) white crystals: mp 42-44°C. ¹H NMR (CDCl₃) δ 1.7 (4H, m), 2.4 (3H, s), 2.4 (2H, m), 2.7 (2H, m), 11.1 (1H, s).

3-Phenyldithio-propanoic acid (4a). To a stirred solution of diphenyl disulfide (3.0 g, 13.8 mmol) in a mixture of ether (10 ml) and methanol (20 ml), under a nitrogen atmosphere, at room temperature was added a solution of 3-mercaptopropanoic acid (**2b**) (0.49 g, 4.6 mmol) in ether (5 ml), followed by a solution of 10 M NaOH (0.46 ml, 4.6 mmol). The reaction mixture was stirred at room temperature for 20 hours, then stripped of the solvents under reduced pressure. The product was purified by column chromatography on silica gel eluting with ethyl acetate/hexane. The product was obtained as a white solid (0.56 g, 56.6%), mp 57-59°C. NMR (CDCl₃, TMS) δ 2.6-3.0 (4H, m), 7.1-7.6 (5H, m), 10.6 (1H, s).

3-(4-Nitrophenyldithio)-propanoic acid (4b). To a stirred solution of bis-(4-nitrophenyl)-disulfide (3.00 g, 9.73 mmol) dissolved in a mixture of THF (200 ml) and methanol (50 ml) was added 3-mercaptopropanoic acid (**2b**) (0.688 g, 6.49 mmol). One drop of a solution of 10 N NaOH was then added to the mixture and the reaction stirred for one hour. The reaction mixture was then diluted with saturated NaCl (100 ml) and extracted with ethyl acetate (3 x 75 ml). The combined organic layers were dried over sodium sulfate, evaporated under reduced pressure and the product was chromatographed over silica gel eluting with methylene chloride/ethyl acetate to yield 0.885 g (53%) of a light yellow solid; mp 98-100°C. ¹H NMR (CD₃COCD₃) δ 2.8 (2H, m), 3.1 (2H, m), 7.8 (2H, d), 8.2 (2H, d).

N-methyl-N-methyldithioacetoyl-L-alanine (5a). To a stirred solution of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (2.99 g, 15.6 mmol) and triethylamine (1.58 g, 15.6 mmol) in dry CH₂Cl₂ (40 ml) at 0°C was added a solution of methyldithioacetic acid (Singh et al, 104 *Anal. Biochem.* 51-58 (1980) (**2a**)) (1.66 g, 12.0 mmol) in dry CH₂Cl₂ (20 ml). A solution of 4-dimethylaminopyridine (0.073 g, 0.60 mmol) in dry CH₂Cl₂ (2 ml) was added and the mixture stirred for 45 min. at 0°C. A mixture of N-methyl-L-alanine (0.619 g, 6.00 mmol) and triethylamine (0.607 g, 6.00 mmol) in dry DMF (30 ml) was then added and the mixture stirred at 0°C for two hours. The reaction mixture was diluted with water (100 ml), stirred for another thirty minutes, then acidified (aqueous HCl) and extracted with ethyl acetate (4 x 75 ml). The combined organic layers were washed several times with water, dried over Na₂SO₄, and evaporated under reduced pressure. The residue was chromatographed over silica gel eluting with methylene chloride/ethyl acetate to yield 0.25 g (19%) of a pale yellow oil. ¹H-NMR (CDCl₃) δ 1.4 (3H, d), 2.4 (3H, s), 2.9, 3.0 (total 3H, 2s), 3.6 (2H, s), 4.7, 5.2 (total 1H, 2q), 9.8 (1H, s).

N-methyl-N-(3-methyldithio-propanoyl)-L-alanine (5b). To a stirred solution of 3-methyldithio-propanoic acid (**3b**) (1.00 g, 6.57 mmol) in dry THF (20 ml) at -10°C under argon was added isobutylchloroformate (0.897g, 6.57mmol) and triethylamine (0.665 g, 6.57 mmol) and the reaction mixture was stirred for 15 minutes. A mixture of N-methyl-L-alanine (0.677 g, 6.57 mmol) and triethylamine (1.33 g, 13.14 mmol) in water (10 ml) was added and the reaction mixture was stirred at room temperature overnight. The mixture was then diluted with water (50 ml), acidified (aqueous HCl), and

extracted with ethyl acetate (4 x 50 ml). The combined organic layers were dried over sodium sulfate, the solvent evaporated under reduced pressure and the residue chromatographed over silica gel eluting with methylene chloride/ethyl acetate to yield 0.556 g (34%) white crystals: mp 98-100°C. ¹H NMR (CDCl₃) δ 1.3 (3H, d), 2.2 (3H, s), 2.7 (4H, m), 4.5 (1H, q), 10.7 (1H, s). Anal. Calculated for C₈H₁₅NO₃S₂: C, 40.49; H, 6.37; N, 5.90; mol wt. 237.33. Found: C, 40.42; H, 6.41; N, 5.93.

N-methyl-*N*-(4-methyldithio-butanoyl)-*L*-alanine (5c). To a stirred solution of 4-methyldithiobutanoic acid (3c) (0.200 g, 1.20 mmol) in dry THF (10 ml) at -20°C under Argon was added isobutyl chloroformate (0.164 g, 1.20 mmol) and triethylamine (0.121 g, 1.20 mmol) and the mixture was stirred for twenty minutes. A mixture of *N*-methyl-*L*-alanine (0.124 g, 1.20 mmol) and triethylamine (0.243 g, 2.40 mmol) in water (5 ml) was then added and the reaction mixture was stirred at room temperature for five hours. The reaction mixture was then treated as described above for 5b giving the title compound as white crystals (0.135 g, 44%): mp 92-93°C. ¹H NMR (CDCl₃) δ 1.4 (3H, d), 2.0 (2H, m), 2.3 (3H, s), 2.7 (4H, m), 2.9 (3H, s), 5.1 (1H, g), 10.5 (1H, s).

N-methyl-*N*-(5-methyldithio-pentanoyl)-*L*-alanine (5d). To a stirred solution of 5-methyldithio-pentanoic acid (3d) (0.202 g, 1.12 mmol) in dry THF (15 ml) at -40°C under Argon was added isobutyl chloroformate (0.153 g, 1.12 mmol) and triethylamine (0.113 g, 1.12 mmol) and the reaction mixture was stirred for 20 minutes at -10°C. A solution of *N*-methyl-*L*-alanine (0.116 g, 1.12 mmol) and triethylamine (0.227 g, 2.24 mmol) in water (5 ml) was then added and the mixture was stirred at 0°C for five hours. The reaction mixture was treated as described above for 5b affording the title compound as white crystals (0.196 g, 66%): mp 84°C. ¹H NMR (CDCl₃) δ 1.4 (3H, d), 1.8 (4H, m), 2.4 (3H, s), 2.7 (4H, m), 3.0 (3H, s), 5.2 (1H, q), 10.7 (1H, s).

N-Methyl-*N*-(3-phenyldithio-propanoyl)-*L*-alanine (5e). A solution of 3-phenyldithio-propanoic acid (4a) (1.8 g, 8.4 mmol) in dry THF was stirred vigorously under a nitrogen atmosphere and cooled to -15°C. Isobutyl chloroformate (1.2 ml, 9.25 mmol) and triethylamine (1.29 ml, 9.25 mmol) were added and the reaction mixture was stirred at this temperature for ten minutes. A solution of *N*-methyl-*L*-alanine (0.87 g, 8.4 mmol) and triethylamine (1.29 ml, 9.25 mmol) in water (10 ml) was then added and the reaction mixture was stirred for fifteen minutes at -15°C and then warmed to room temperature and stirred for an additional period of 2.5 hours. 1 M HCl (10 ml) was added and the reaction mixture was extracted with ethyl acetate (4 x 50 ml). The combined organic layers were dried with Na₂SO₄, filtered, and evaporated under reduced pressure. The crude mixture was purified by column chromatography on silica gel eluting with ethyl acetate/hexane-containing 2% acetic acid to give a white solid (1.5 g, 60%): mp 96-97°C. NMR (CDCl₃/TMS) δ 1.4 (2H, d), 2.7-3.0 (7H, m), 5.2 (1H, q), 7.2-7.6 (5H, m).

N-Methyl-*N*-[3-(4-Nitrophenyldithio)-propanoyl]-*L*-alanine (5f). To a stirred solution of 3-(4-nitrophenyldithio)-propanoic acid (4b) (0.100 g, 0.386 mmol) in dry THF (10 ml) at -40°C under argon was added isobutyl chloroformate (0.053 g, 0.386 mmol) and triethylamine (0.039 g, 0.38 mmol) and the reaction stirred at 0°C for sixty minutes. An aqueous solution (5 ml) of *N*-methyl-*L*-alanine (0.040 g, 0.386 mmol) and triethylamine (0.039 g, 0.386 mmol) was then added and the mixture stirred at 0°C for five hours. The mixture was diluted with water (50 ml), acidified (aqueous HCl), and extracted with ethyl ether (3 x 25 ml). The combined organic layers were dried (Na₂SO₄), and the solvent evaporated under reduced pressure. The residue was chromatographed over silica gel eluting with methylene chloride/ethyl acetate to yield 0.048 g (36%) yellow crystals: mp 74-77°C. NMR (CDCl₃) δ 1.4 (3H, d), 2.6-3.4 (4H, m), 2.9 (3H, s), 5.1 (1H, q), 7.6-8.3 (4H, 2d).

Esterification of Maytansinol. In a typical experiment, a solution of the carboxylic acid 5 (133 μmols) in dry methylene chloride (0.3 ml), was stirred under an argon atmosphere, and treated sequentially with solutions of dicyclohexylcarbodiimide (160 μmol) in methylene chloride (0.2 ml), 1 M ZnCl₂ in ether (26.2 μmol) and maytansinol (15 mg, 26.6 μmol) in methylene chloride (0.3 ml). The reaction mixture was stirred at room temperature for three hours, and then filtered and the filtrate evaporated under reduced pressure. Preparative TLC of the crude mixture on silica gel, eluting twice with 6% methanol in CHCl₃ gave two major UV absorbing band. Both bands were isolated by extraction with ethyl acetate and characterized by NMR spectroscopy.

Esterification of Maytansinol with *N*-methyl-*N*-(methyldithio-propanoyl)-*L*-alanine. A solution of the carboxylic acid 5b (31.5 mg., 133 μmol) in dry CH₂Cl₂ (0.3 ml) was stirred under an argon atmosphere and treated sequentially with solutions of dicyclohexylcarbodiimide (33 mg, 160 μmol) in CH₂Cl₂, 1 M ZnCl₂ in ether (26.6 μmol), and maytansinol (15 mg, 26.6 μmol) in CH₂Cl₂ (0.3 ml). The reaction mixture was stirred at room temperature for three hours and then filtered and the solvent evaporated under reduced pressure. Preparative TLC on silica gel, eluting twice with 6% methanol in CHCl₃ gave two strong UV absorbing bands with R_f values of 0.6 and 0.7 respectively. Both bands were isolated by extraction with ethyl acetate. NMR spectra and cytotoxicity analyses confirmed that the higher R_f band was the *D*-aminoacyl ester (45%) while the lower band was the desired *L*-aminoacyl ester 6b (55%). Both bands were further purified by HPLC on a Waters Radialpak C-18 column, eluting at a flow rate of 1.5 ml/min with a linear gradient of acetonitrile in H₂O (55% CH₃CN to 80% CH₃CN, over 10 min). Under these conditions both isomers had an identical retention time of 7.3 min. NMR (CDCl₃) *L*-aminoacyl isomer: δ 0.84(3H, s), 1.11-1.23 (1H, m), 1.31 (3H, d, J = 6Hz), 1.35 (3H, d, J = 7Hz), 1.46-1.52 (1H, m), 1.68 (3H, s), 1.97 (1H, d, J = 9Hz), 2.24 (1H, dd, J = 12Hz and 15Hz), 2.30 (3H, s), 2.65 (1H, dd, J = 12Hz and 15Hz), 2.73-2.86 (2H, m), 2.90 (3H, s), 2.92-3.03 (2H, m), 3.08 (1H, d, J = 9Hz), 3.14 (1H, d, J = 12Hz), 3.28 (3H, s), 3.39 (3H, s), 3.54 (1H, d, J = 9Hz), 3.72 (1H, d, J = 13Hz), 4.02 (3H, s), 4.31 (1H,

t, J = 11Hz), 4.82 (1H, dd, J = 3Hz and 12Hz), 5.45 (1H, q, J = 7Hz), 5.69 (1H, dd, J = 9Hz and 15Hz), 6.25 (1H, s), 6.47 (1H, dd, J = 11Hz and 15Hz), 6.67 (1H, d, J = 1.5Hz), 6.77 (1H, d, J = 11Hz), 6.85 (1H, d, J = 1.5Hz).

Esterification of Maytansinol with *N*-methyl-*N*-(methyl-dithiobutanoyl)-*L*-alanine. A solution of the carboxylic acid **5c** (8.9 mg, 35.5 μ mol) in CH_2Cl_2 (0.15 ml) was stirred under an atmosphere of argon, and treated sequentially with solutions of DCC (8.8 mg, 42.6 μ mol) in CH_2Cl_2 , 1M ZnCl_2 (7.1 μ mol) in ether and maytansinol (4.0 mg, 7.1 μ mol) in CH_2Cl_2 . The reaction mixture was stirred at room temperature for 3 hours, and then filtered and the filtrate was evaporated under reduced pressure. The residue was purified by preparative TLC on silica gel, eluting twice with 7% methanol in CHCl_3 . Two new UV absorbing bands (R_f = 0.65 and 0.75) were obtained. The products were isolated by extraction with ethyl acetate. The higher R_f band was determined to be the *D*-aminoacyl ester **6d** (41%), while the lower R_f band was the desired *L*-aminoacyl ester **6c** (59%). Both isomers were further purified by HPLC using a Waters Radialpak C-18 column, eluting at a flow rate of 2 ml/min, with a linear gradient of acetonitrile in water (50% to 80% acetonitrile, over 10 minutes). Under these conditions the *D*-aminoacyl ester had a retention time of 7.4 minutes, while the *L*-aminoacyl isomer had a retention time of 7.6 min.

Esterification of Maytansinol with *N*-methyl-*N*-(phenyldithio-propanoyl)-*L*-alanine. A solution of the carboxylic acid **5e** (31.5 mg, 105 μ mol) in CH_2Cl_2 (0.4 ml) was stirred under argon and treated sequentially with solutions of DCC (26 mg, 126 μ mol) in CH_2Cl_2 , 1 M ZnCl_2 (17.7 μ mol) in ether and maytansinol (10 mg, 17.7 μ mol) in CH_2Cl_2 (0.2 ml). The reaction mixture was stirred at room temperature for three hours. The precipitate was removed by filtration and the filtrate concentrated under reduced pressure. Preparative TLC of the residue on silica gel, eluting twice with 5% methanol in CHCl_3 gave two strongly UV absorbing bands with R_f values of 0.5 and 0.6. The two products were isolated by extraction with ethyl acetate and characterized by NMR spectroscopy. The higher R_f band was found to be the *D*-aminoacyl ester **6f** (45%) of maytansinol and the lower band was the *L*-aminoacyl ester **6e** (55%). The overall yield of ester from maytansinol was 44%. The products were further purified by HPLC on a Waters Radialpak C-18 column, eluting at a flow rate of 1.5 ml/min, with a linear gradient of acetonitrile in water (70% to 90% acetonitrile, over 10 min). Under these conditions both isomers had an identical retention time of 6.0 min. NMR (CDCl_3) *L*-aminoacyl isomer **6e**: δ 0.82 (3H, s), 1.11-1.25 (1H, m), 1.33 (3H, d, J = 3Hz), 1.61 (3H, s), 1.63 (3H, d, J = 14Hz), 2.19 (1H, dd, J = 13Hz and 15Hz), 2.61 (1H, dd, J = 12Hz and 15Hz), 2.78 (3H, s), 2.68-3.03 (2H, m), 3.07 (1H, d, J = 9Hz), 3.20 (3H, s), 3.38 (3H, s), 3.53 (1H, d, J = 9Hz), 3.63 (1H, d, J = 13Hz), 3.68 (3H, s), 4.01 (3H, s), 4.30 (1H, t, J = 11Hz), 4.79 (1H, dd, J = 3Hz and 8 Hz), 5.43 (1H, q, J = 7Hz), 5.68 (1H, dd, J = 9Hz and 15Hz), 6.23 (1H, s), 6.45 (1H, dd, J = 12Hz and 15Hz), 6.60 (1H, d, J = 1.5 Hz), 6.75 (1H, d, J = 12Hz), 6.77 (1H, d, J = 1.5Hz), 7.22-7.40 (5H, m).

Reduction of Maytansinoids. The disulfide-containing esters **6** of maytansinol were reduced to the thiol-containing maytansinoids **7** with dithiothreitol by the methods outlined below. In all cases the reactions were performed at 4°C and the products were purified by HPLC.

Reduction of Maytansinoid (6b). A solution of the methyl disulfide ester **6b** (0.89 μ mol) in ethanol (0.23 ml) and 0.1 M potassium phosphate buffer pH 7.5 (0.16 ml) containing 1 mM EDTA was cooled in ice, under an argon atmosphere and treated with a solution of 100 mM dithiothreitol (1.33 μ mol). The progress of reduction was followed by HPLC and judged to be complete after ten hours. The thiol-containing maytansinoid **7b** was purified by HPLC using a Waters Radialpak C-18 column at a flow rate of 1.5 ml/min, eluting with a linear gradient of acetonitrile in water (55%-80% acetonitrile, over 10 min). Under these conditions the product eluted as a single peak with a retention time of 5.4 minutes. The presence of one mole of sulfhydryl group/mol product was confirmed using Ellman's assay. The product was further characterized by NMR spectroscopy. NMR (CDCl_3): δ 0.84 (3H, s), 1.33 (3H, d, J = 5Hz), 1.35 (3H, d, J = 5Hz), 1.60 (3H, s), 1.68 (3H, s), 2.22 (1H, dd, J = 3Hz and 14Hz), 2.60-2.82 (2H, m), 2.88 (3H, s), 3.08-3.20 (2H, m), 3.25 (3H, s), 3.39 (3H, s), 3.55 (1H, d, J = 9Hz), 3.71 (1H, d, J = 12Hz), 4.02 (3H, s), 4.32 (1H, t, J = 10Hz), 4.81 (1H, dd, J = 3Hz and 12Hz), 5.45 (1H, q, J = 7Hz), 5.67 (1H, dd, J = 9Hz and 15Hz), 6.25 (1H, s), 6.47 (1H, dd, J = 11 Hz and 15Hz), 6.70 (1H, d, J = 1.5Hz), 6.75 (1H, d, J = 11Hz), 6.86 (1H, d, J = 1.5Hz).

Reduction of Maytansinoid (6e). A solution of the phenyldisulfide ester **6e** (0.463 μ mol) in ethanol (0.22 ml) and 0.1 M potassium phosphate buffer, pH 7.5 (0.18 ml) containing 2 mM EDTA was cooled in ice and treated with a solution of 20 mM dithiothreitol (0.69 μ mol, 0.035 ml). The progress of the reaction was monitored by HPLC and judged to be complete in 40 minutes. The thiol-containing maytansinoid generated in this manner was purified as described above and found to be identical to **7b**.

EXAMPLE 2

In Vitro Cytotoxicity Assays

The disulfide-containing maytansinoid drugs of the invention were evaluated for their ability to suppress proliferation of various human tumor cell lines *in vitro*. Two adherent cell lines KB (human epidermoid carcinoma) and SKBR3 (human breast tumor) and the non-adherent cell line, Namalwa (Burkitt's lymphoma) were used for the assessment of cytotoxicity of these compounds. Cells were exposed to the compounds for 24 hours and the surviving fractions of cells were measured in direct assays. (KB and SKBR3 were assayed for plating efficiency (Goldmacher et al, 102 *J. Cell*.

Biol. 1312-1319 (1986) and Namalwa was assayed by growth back-extrapolation (Goldmacher et al, 135 *J. Immunol.* 3648-3651 (1985)). IC_{50} values were then calculated from these data. The results of these tests are presented in Table 1.

Table 1

Cytotoxicity of New Maytansinoid Derivatives IC_{50} (M) values for 24 hour exposure			
Compound	Cell Line		
	KB	SKBR3	NAMALWA
<u>1a</u>	3.4×10^{-11}	1.1×10^{-10}	4×10^{-10}
<u>6b</u>	1.1×10^{-11}	1.1×10^{-11}	6.4×10^{-9}
<u>6c</u>	8.5×10^{-12}	3.8×10^{-11}	3.2×10^{-9}
<u>6d</u>	1.9×10^{-10}	nd	1.3×10^{-8}
<u>6e</u>	1.0×10^{-10}	nd	6×10^{-10}
<u>6f</u>	2.0×10^{-9}	nd	8×10^{-9}
<u>7b</u>	6×10^{-10}	$< 10^{-11}$	1×10^{-8}
<u>7c</u>	4×10^{-10}	nd	nd

The *L*-aminoacyl isomers 6b and 6c are highly toxic towards KB and SKBR3 cells with IC_{50} values of $\leq 1 \times 10^{-11}$ M while the corresponding phenyl-dithio analog 6e has an IC_{50} value of 1×10^{-10} M on KB cells. The methyl-dithio analogs 6b and 6c are 3-10 fold more toxic than maytansine which has an IC_{50} of 3×10^{-11} M and 1×10^{-10} M to KB and SKBR3 cells respectively. These two compounds, however, are somewhat less toxic, $IC_{50} \geq 3 \times 10^{-9}$ M, than maytansine, $IC_{50} = 4 \times 10^{-10}$ M, to Namalwa cells while the phenyldithio compound 6e is almost as toxic, $IC_{50} = 6 \times 10^{-10}$ M, as maytansine. The *D*-aminoacyl isomers 6d and 6f were at least 20 times less toxic than their *L* counterparts, 6c and 6e, to all three cell lines. The free thiol-containing maytansinoids are also very toxic to KB and SKBR3 cells with IC_{50} values for 7b of 6×10^{-10} M and $< 10^{-11}$ M respectively.

Since the methyl-dithio-maytansinoids are even more toxic than maytansine to solid tumor cell lines like KB and SKBR3, a wider panel of solid tumor cell lines was tested in a manner analogous to that described above to see whether this was a general phenomenon. The results are summarized in Table 2.

Table 2

IN VITRO CYTOTOXICITY OF MAYTANSINE (<u>1a</u>) AND MAYTANSINOID DERIVATIVE (<u>6b</u>) TOWARDS DIFFERENT CELL LINES		
CELL LINE	IC_{50} (24hr.exposure) Molar	
	Maytansine	May-CH ₂ CH ₂ SSMe(<u>6b</u>)
KB (Epidermoid Carcinoma)	3.4×10^{-11}	1.1×10^{-11}
SKBR3 (Breast adenocarcinoma)	1.1×10^{-10}	1.2×10^{-11} (1×10^{-13} , 72hr exposure)
SW620 (Colon adenocarcinoma)	$> 10^{-10}$	4×10^{-11}
A498 (Renal carcinoma)	$> 10^{-10}$	1×10^{-10}
Ovar3 (Ovarian carcinoma)	1.0×10^{-10}	5×10^{-11}
Namalwa (Burkitt's lymphoma)	4×10^{-10}	6×10^{-9}
HL60(Promyelocytic leukemia)	5×10^{-10}	8×10^{-10}

The methyl-dithio compound 6b was very toxic to SW620 (colon adenocarcinoma) and A498 cells (renal carcinoma) with IC_{50} values of 4×10^{-11} M and 1×10^{-10} M respectively, while maytansine was nontoxic (surviving fraction = 100%)

even at 1×10^{-10} M to these cell lines. Compound **6b** was twice as cytotoxic, $IC_{50} = 5 \times 10^{-11}$ M, as maytansine to the ovarian cancer cell line OVCAR3. The high cytotoxicity of the methyl-dithio maytansinoids to adherent cell lines is an unexpectedly superior property of these compounds and indicates that cancers caused by solid tumors are good targets for treatment with conjugates of these compounds with appropriate cell targeting agents.

EXAMPLE 3

Conjugation of Antibodies

Conjugation of Maytansinoid **7b** to Antibodies via Disulfide links: The conjugation of the thiol-containing maytansinoid **7b** to antibodies via disulfide links was performed in two steps. In the first step dithiopyridyl groups were introduced into antibodies using succinimidyl pyridyldithiopropionate (SPDP) as described by Carlsson et al. The thiopyridyl groups were then displaced by reaction with the maytansinoid **7b** to produce a conjugate.

Preparation of Antibody-SS-Maytansinoid Conjugates. Antibodies anti-B4, anti-T9 and A7 were modified with SPDP as described in the literature. Between 1 to 10 dithiopyridyl groups were introduced on the average per antibody molecule.

A solution of the dithiopyridyl modified antibody at a concentration of 1 mg/ml in 0.1 M potassium phosphate buffer pH 7.0 containing 1 mM EDTA at 25°C was treated with the thiol-containing maytansinoid **7b** (1.25 molar equivalent/dithiopyridyl group). The release of pyridine-2-thione from the modified antibody was monitored spectrophotometrically at 343 nm and was found to be complete in 30 minutes. The antibody-maytansinoid conjugate was purified and freed of unreacted drug and other low molecular weight material by gel filtration through a column of Sephadex G-25. The number of maytansinoid molecules bound per antibody molecule was determined by measuring the ratio between the absorbances at 252 nm and 280 nm. An average of 1-10 maytansinoid molecules/antibody molecule can be linked via disulfide bonds by this method.

Conjugation of Maytansinoid **7b** to Antibodies via a Noncleavable Thioether Link: The conjugation of the thiol-containing maytansinoid **7b** was performed in two steps. The antibody was first reacted with succinimidyl maleimidomethylcyclohexane carboxylate (SMCC) to introduce maleimido groups. The modified antibody was then reacted with maytansinoid **7b** forming thioether links.

Preparation of Antibody-Maytansinoid Conjugates (non-cleavable). Antibodies, anti-B4, anti-T9 and A7 were modified with SMCC as described in the literature.

The modified antibodies were treated with maytansinoid (**7b**) (1.25 molar equivalent/maleimido group). The mixtures were incubated overnight at 4°C. The antibody-maytansinoid conjugates were purified as described above. Typically, an average of 1-10 maytansinoid molecules/antibody molecule were linked.

EXAMPLE 4

Other Methods of Linking Maytansinoids

Acid Labile Linker

Maytansinol can be esterified with *N*-methyl-*N*-(3-*t*-boc-aminopropanoyl)-*L*-alanine in the presence of dicyclohexylcarbodiimide and zinc chloride as described for **6b**. Cleavage of the *t*-boc protecting group with trifluoroacetic acid will give a C-3 ester of maytansinol containing a terminal amino group. This amino group containing maytansinoid derivative can be linked to antibodies and other cell binding agents via an acid labile linker as previously described (Blattler et al, 24 *Biochemistry*, 1517-1524 (1985), U.S. Patent Nos. 4,542,225, 4,569,789 and 4,764,368).

Photolabile Linker

The amino group-containing maytansinoid derivative described above can be linked to cell binding agents via a photolabile linker as previously described (Senter et al, 42 *Photochemistry and Photobiology*, 231-237 (1985), U.S. Patent 4,625,014).

Peptidase Labile Linker

The amino group-containing maytansinoid described above can also be linked to cell binding agents via peptide spacer linkers. It has been previously shown that short peptide spacers between drugs and macromolecular protein carriers are stable in serum but are readily hydrolyzed by intracellular lysosomal peptidases (Trouet et al, 79 *Proc. Nat'l. Acad. Sci.*, 626-629 (1982)). The amino group-containing maytansinoid can be condensed with peptides such as Ala-

Leu, Leu-Ala-Leu and Ala-Leu-Ala-Leu using condensing agents such as 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide-HCl to give a peptide derivative of the maytansinoid which can then be linked to cell binding agents.

Esterase Labile Linker

Maytansinol can be esterified with *N*-methyl-*N*-(3-*tert*-butoxy-propanoyl)-*L*-alanine in the presence of dicyclohexylcarbodiimide and zinc chloride as previously described for **6b**. Cleavage of the *tert*-butyl protecting group with trifluoroacetic acid gives a C-3 ester of maytansinol containing a terminal primary hydroxyl group. This maytansinoid derivative can be succinylated with succinic anhydride and then linked to a cell binding agent to produce a conjugate that can be cleaved by intracellular esterases to liberate free drug. (For examples see: Aboud-Pirak et al, 38 *Biochem. Pharmacol.*, 641-648 (1989), Laguzza et al, 32 *J. Med. Chem.*, 549-555 (1989)).

Disulfide Link Through Thiol of *N*-Methyl Cysteine Or *N*-Methyl-Homocysteine

The disulfide-containing esters **10** of maytansinol can be reduced with dithiothreitol as described for **7b** to produce the thiol-containing maytansinoids **11** which can be conjugated to cell binding agents via disulfide or thioether links as previously described (See Example 3).

Methyldithio-*N*-Methylcysteine (8): A solution of *N*-methylcysteine (Undhein, K., & Eidem, A., 23 *Acta Chem. Scandinavica* 3129-3133 (1970) (1.5 g, 11.1 mmol) in H₂O (20 ml) was stirred at room temperature, under an atmosphere of argon, and treated with a solution of methyl methanethiol sulfonate (3.0 ml, 29.2 mmol) in ethanol (10 ml). The reaction mixture was stirred at this temperature for two hours and then diluted with H₂O (100 ml) and washed with ether (4 x 40 ml). The aqueous layer was acidified to pH 2 and passed through an Amberlite IRA 93 (-OH form) column. The column was washed with water and the effluent was collected and evaporated to dryness under reduced pressure to give a white solid (1.2g, 60%), mp 194-5°C. NMR (D₂O, TMS ext. standard): δ 2.2(3H,s), 2.5(3H,s), 3.2(2H,d), 3.7(1H,q).

***N*-Acetyl-*N*-Methyl-Methyldithiocysteine (9):** To a solution of glacial acetic acid (0.25 ml, 4.4 mmol) in dry THF (4 ml), at -20°C under an atmosphere of N₂ were added, with stirring, isobutylchloroformate (0.57 ml, 4.4 mmol) and triethylamine (0.61 ml, 4.4 mmol). The reaction mixture was stirred at this temperature for 20 minutes and then treated with a solution of methyldithio-*N*-methylcysteine (0.4 g, 2.2 mmol) and triethylamine (0.45 ml, 3.3 mmol) in H₂O and then allowed to stir overnight. The mixture was then diluted with water (25 ml) and acidified to pH 2 with HCl and extracted with ethyl acetate (4 x 50 ml). The combined organic layer was dried with sodium sulfate and the solvent evaporated under reduced pressure to give the product as a pale yellow solid (0.2g, 55%) mp 137-8°C NMR (CDCl₃): 2.1(3H,s), 2.3(3H,s), 3.0(3H,s), 3.2(2H,d) and 4.8(1H,q).

Esterification Of Maytansinol with *N*-Acetyl-*N*-Methyl Methyldithiocysteine: A solution of compound **9** (15.6 mg, 0.07 mmol) in dry methylene chloride (0.45 ml) was stirred at room temperature under an argon atmosphere and treated sequentially with solutions of 1 M ZnCl₂ in ethyl ether (0.028 mmol), DCC (17.3 mg, 0.084 mmol) in methylene chloride (0.2 ml), and maytansinol (4.0 mg, 0.007 mmol) in CH₂Cl₂ (0.1 ml). The reaction mixture was stirred for 3 hours and then filtered and the filtrate evaporated under reduced pressure. The residue was purified by preparative TLC on silica gel eluting with 6% methanol in CHCl₃. The product was isolated by extraction with ethyl acetate and further purified by HPLC on a Waters Radialpak C-18 column eluting with a linear gradient of acetonitrile in water (55-80% acetonitrile/10 min) at a flow rate of 1.5 ml/min. The *L*-aminoacyl ester **10a** and the *D*-aminoacyl ester **10b** of maytansinol that were obtained by this method were readily separable with retention times of 5.0 and 5.8 minutes respectively.

EXAMPLE 5

Cell Cultures and In Vitro Cytotoxicity Assays

Cells of the human promyelocytic leukemia cell line, HL-60 (ATCC CCL 240) and the Burkitt's lymphoma cell line Namalwa (ATCC CRL 1432) were grown as suspension cultures in RPMI-1640 medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. All other cell lines described below were grown as adherent cultures. Human epidermoid carcinoma cell line, KB (ATCC CCL 17), human renal carcinoma cell line A498 (ATCC HTB 44), human colon adenocarcinoma cell lines SW620 (ATCC CCL 227) and HT-29 (ATCC HTB 38) were grown in RPMI-1640 medium supplemented with 10% fetal calf serum and 2 mM L-glutamine. Human breast carcinoma SKBR3 cells (ATCC HTB 30) were grown in DMEM supplemented with 15% fetal calf serum containing 2 mM glutamine and the human ovarian adenocarcinoma cell line OVCAR3 (ATCC HTB 161) was grown in RPMI-1640 medium supplemented with 10% fetal calf serum containing 10 μ g/ml insulin and 2 mM L-glutamine.

Three different antibodies were used for conjugation via disulfide links to thiol-containing maytansinoids. In all these experiments the maytansinoid derivative **7b** was used for conjugation. Conjugates were prepared with the antibodies anti-B4, which is against the B cell antigen CD19; anti-T9 (5E9) which is an anti-human transferrin receptor antibody, and A7, which is an anti-human colon cancer antibody.

Cytotoxicity assays were performed in the respective media described above. The cytotoxicity of the maytansinoids and their antibody conjugates to HL-60 and Namalwa cells was measured by back-extrapolation of cell proliferation curves. Cytotoxicity of these compounds to the rest of the cell lines was determined using a clonogenic assay as previously described.

The conjugates were assessed for *in vitro* cytotoxicity and the IC_{50} values for cell lines Namalwa, KB, HT-29 and SW620 are summarized in Table 3.

TABLE 3

IN VITRO CYTOTOXICITY AND SPECIFICITY OF ANTIBODY-MAYTANSINOID CONJUGATES		
CELL LINE	CONJUGATE	IC_{50} (Molar) (24 h exposure)
Namalwa	anti-B4-SS-May	7×10^{-9}
	anti-T9-SS-May	2×10^{-9}
KB	anti-T9-SS-May	2×10^{-10}
	anti-T9-May (non cleavable)	4×10^{-9}
	A7-SS-May	8×10^{-9} (Non-Binding Antibody, 72 hr Exp.)
HT-29	A7-SS-May	1.5×10^{-10}
		4×10^{-11} (72 hr. Exp.)
SW620	A7-SS-May	1.6×10^{-10}

The disulfide-linked anti-B4-maytansinoid conjugate (anti-B4-SS-May) and the anti-T9-drug conjugate (anti-T9-SS-May) were both cytotoxic to Namalwa cells ($IC_{50} = 7 \times 10^{-9}$ M and 2×10^{-9} M, respectively). The disulfide-linked anti-T9 conjugate is even more toxic to KB cells with an IC_{50} value of 2×10^{-10} M. In order to prove the importance of an intracellularly cleavable disulfide link between antibody and drug, a conjugate in which the maytansinoid was linked to the antibody via a non-cleavable thioether bond was prepared. Thus an anti-T9-drug conjugate linked in such a non cleavable fashion was at least 20 times less toxic, ($IC_{50} = 4 \times 10^{-9}$ M) than the corresponding disulfide linked conjugate.

The A7-maytansinoid conjugate is highly toxic to the colon adenocarcinoma cell lines HT-29 and SW620. The IC_{50} for these cell lines after a 24 hour exposure to the conjugate were 1.5×10^{-10} M and 1.6×10^{-10} M, respectively. The cytotoxicity after a 72 hour exposure was even more dramatic ($IC_{50} = 4 \times 10^{-11}$ M on HT-29 cells). Several experiments were designed to demonstrate *in vitro* specificity of these immunoconjugates. (Some of the data is shown graphically in Fig. 5). First, addition of an excess of unconjugated A7 antibody completely abolishes cytotoxicity of the A7-maytansinoid conjugate to HT-29 and SW620 cells at both 24 and 72 hour exposures. This observation also indicates the absence of free drug in the conjugate. Second, the conjugate prepared above with the antibody anti-B4, which does not bind to HT-29 and SW620 cells, is much less cytotoxic to these cells than the A7 conjugate. Another independent experiment that establishes specificity is the 100 fold lower cytotoxicity of the A7-maytansinoid conjugate to antigen-negative KB cells, ($IC_{50}^{KB} = 8 \times 10^{-9}$ M vs. $IC_{50}^{HT-29} = 4 \times 10^{-11}$ M, 72 hr Exp.).

EXAMPLE 6

Determination of Specific Affinity of A7-Maytansinoid Conjugates

The specific affinities of disulfide-linked A7-maytansinoid conjugates were analyzed by competition binding assays. The competition of binding of FITC-labeled A7 antibody to HT-29 and SW620 cells by unlabeled A7 and its maytansinoid conjugates were determined by direct immunofluorescence on a Becton-Dickinson FACS. The two cell lines were grown as adherent cells in tissue culture grade flasks containing Dulbecco's modified minimum essential medium with 15% fetal bovine calf serum. The cells were then trypsinized and incubated in suspension, at 37°C, for 30 minutes in the same medium in non-tissue culture grade flasks to prevent adherence of cells to the plastic. The cells were then transferred to wells of 96 well plates and resuspended in minimum essential medium containing 25% pooled human serum. Cell suspensions (0.2 ml suspension containing 100,000 cells/well) were incubated with 6 nM FITC-labeled A7, at the indicated concentrations of unlabeled antibody or maytansinoid conjugates for 1 hour at 0°C. The cells were then washed once with buffer and fixed with 1% formaldehyde in phosphate buffered saline. Mean cell fluorescence was measured on a FACS.

The results are shown in Figures 6(A) and 6(B). Figures 6(A) and 6(B) show that A7-maytansinoid conjugates containing between 3 to 6 drug molecules/antibody molecule bind as well as unconjugated antibody to HT-29 or SW620 cells. This indicates that the present method of conjugation of the maytansinoids to antibodies does not cause any impairment of binding to target cells.

EXAMPLE 7

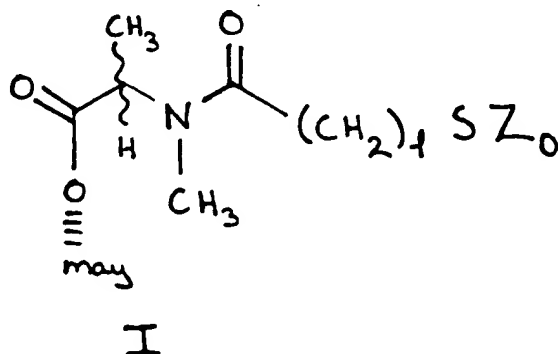
In Vivo Clearance Studies and Pharmacokinetics

Blood clearances of a typical ^{125}I -labelled murine IgG₁ antibody and of its corresponding ^{125}I -labelled maytansinoid conjugate, containing an average of 4 drug molecules/antibody molecule, were determined in female CD-1 mice. The antibody and the maytansinoid conjugates had been radiolabelled by the method of Bolton and Hunter (133 *Biochem. J.* 529-539 (1973)). Greater than 99% of the radioactivity in the samples was bound to protein. The antibody and conjugates (2.7×10^5 cpm each) were injected separately i.v. into the tail vein. Heparinized blood samples were collected from the retroorbital venus plexus at the indicated times and measured for radioactivity content. The results are summarized in Figure 7. The antibody and its maytansinoid conjugate display very similar biphasic clearance profiles.

The invention is of use in killing cell populations which cause cancer, melanomas or graft versus host disease (GVHD). The invention can be used to kill cell infected with bacteria.

Some features of the invention are set out in the following numbered clauses:

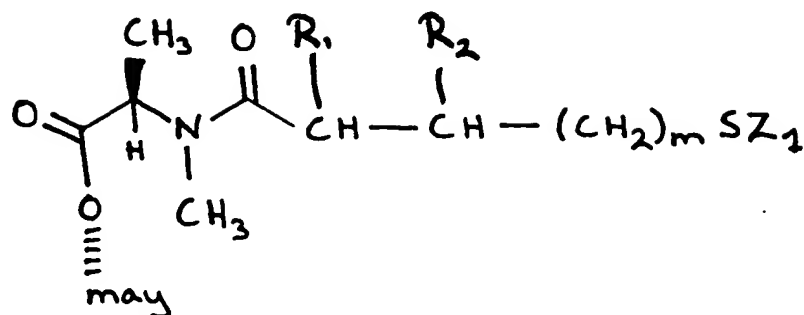
1. A cytotoxic agent comprising one or more maytansinoids chemically linked to a cell binding agent.
2. The cytotoxic agent of clause 1, wherein the maytansinoid is a maytansinoid ester.
3. The cytotoxic agent of clause 2, wherein the maytansinoid ester is a C-3 maytansinoid ester.
4. The cytotoxic agent of clause 2 wherein the maytansinoid ester is an N-methyl-alanine-containing maytansinoid ester.
5. The cytotoxic agent of clause 4, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (I)



wherein:

- Z_0 represents H or SR, wherein R represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic;
 ℓ represents an integer of 1 to 10 and;
 may represents a maytansinoid.

6. The cytotoxic agent of clause 4, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (II):

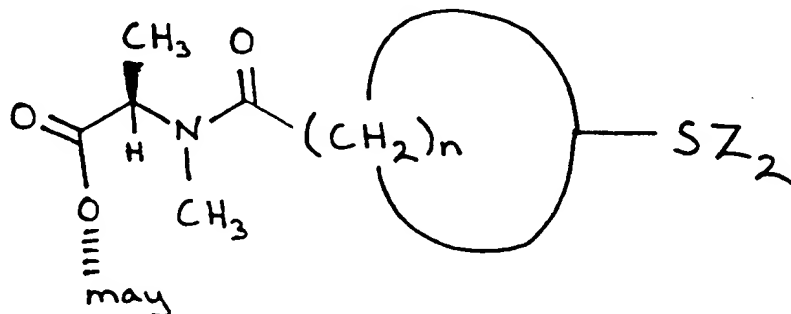


II

wherein:

R_1 and R_2 , which may be the same or different, represents H, CH_3 or CH_2CH_3 ;
 Z_1 represents H or SR_3 , wherein R_3 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;
 m represents 0, 1, 2 or 3; and
 may represents a maytansinoid.

7. A cytotoxic agent of clause 4, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (III):



III

wherein:

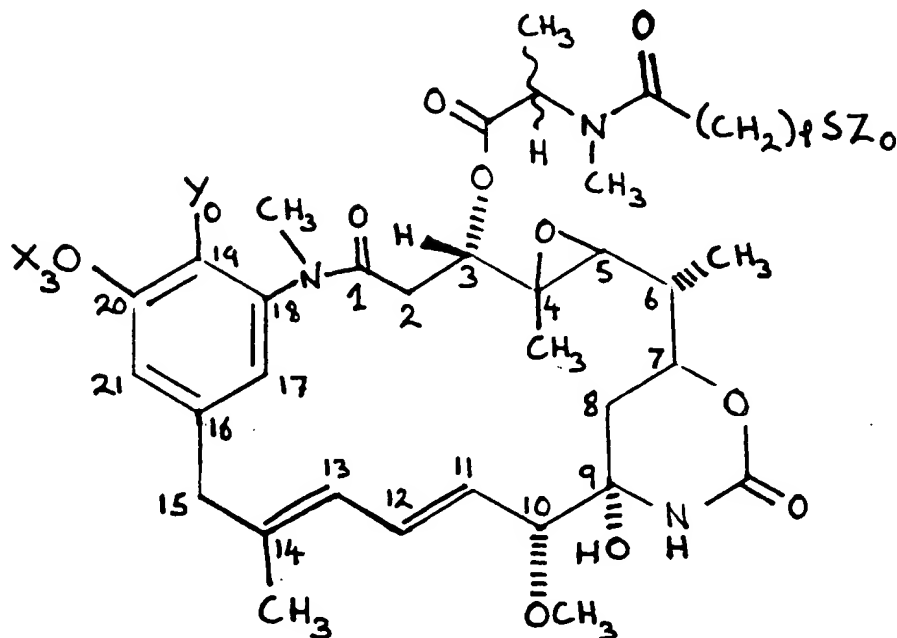
Z_2 represents H or SR_4 wherein R_4 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl, or heterocyclic;
 n represents an integer of 3 to 8; and
 may represents a maytansinoid.

8. The cytotoxic agent of clause 4, wherein the N-methyl-alanine-containing maytansinoid ester is a C-3 ester of maytansinol.

9. The cytotoxic agent of clause 8, wherein the maytansinoid ester is an ester of an analogue of maytansinol that has a modification at C-19 or C-20.

10. The cytotoxic agent of clause 8, wherein the maytansinoid ester is an ester of dechloro maytansinol.

11. The cytotoxic agent of clause 8, wherein the C-3 ester of maytansinol is represented by formula (IV):



IV

wherein:

Z_0 represents H or SR, wherein R represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic;

l represents 1, 2 or 3

Y_0 represents Cl or H; and

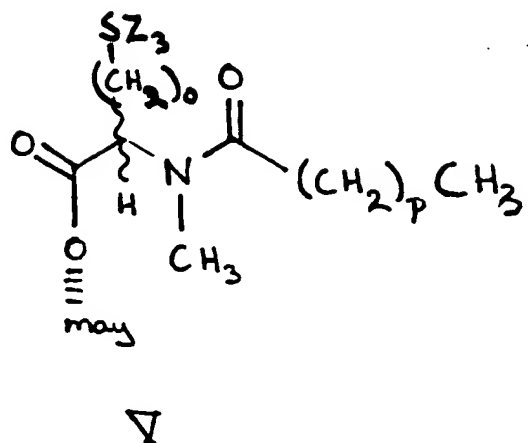
X_3 represents H or CH_3 .

12. The cytotoxic agent of clause 11, wherein R represents methyl.

13. The cytotoxic agent of clause 8, which comprises N-methyl-N-(3-methyldithiopropionyl)-L-alanine.

14. The cytotoxic agent of clause 2 wherein the maytansinoid ester is an N-methyl-cysteine-containing maytansinoid ester.

15. The cytotoxic agent of clause 14, wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (V):



wherein:

Z_3 represents H or SR_5 , wherein R_5 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, sample or substituted aryl, or a heterocyclic;

o represents 1, 2 or 3;

p represents 0 or an integer of 1 to 10; and

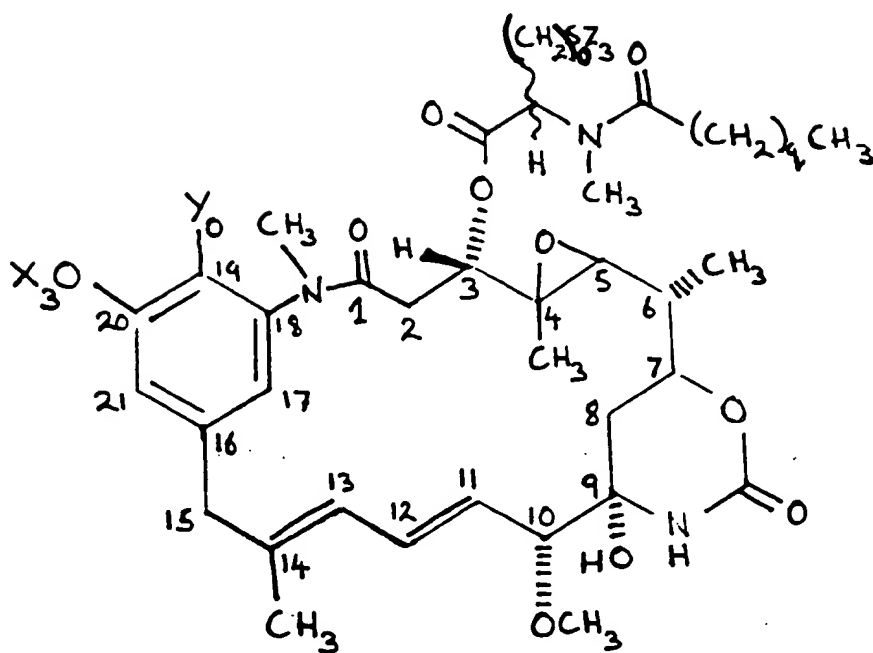
may represents a maytansinod.

16. The cytotoxic agent of clause 14, wherein the N-methyl-cysteine-containing ester is a C-3 ester of maytansinol.

17. The cytotoxic agent of clause 16, wherein the maytansinoid ester is an ester of an analogue of maytansinol that has a modification at C-19 or C-20.

18. The cytotoxic agent of clause 16, wherein the maytansinoid ester is an ester of dechloro maytansinol.

19. The cytotoxic agent of clause 14, wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (VI):



VI

wherein:

Z_3 represents H or SR_5 , wherein R_5 represents methyl, linear alkyl, branched alkyl, cyclic alkyl, simple or substituted aryl or heterocyclic;

o represents 1, 2 or 3;

q represents 0 or an integer of 1 to 10;

Y_0 represents Cl or H; and

X_3 represents H or CH_3 .

20. The cytotoxic agent of clause 19, wherein R_5 represents methyl.

21. The cytotoxic agent of clause 16, which comprises N-acetyl-N-methyl-methyldithiocysteine.

22. The cytotoxic agent of clause 1, wherein the maytansinoid is linked to the cell binding agent through a linking group comprising a disulfide group or a thioether group.

23. The cytotoxic agent of clause 1, wherein the maytansinoid is linked to the cell binding agent through a linking group comprising an acid-labile group, a photolabile group, a peptidase-labile group, or an esterase-labile group.

24. The cytotoxic agent of clause 1, wherein said cell binding agent is a peptide or a non-peptide.

25. The cytotoxic agent of clause 1, wherein said cell binding agent is a polyclonal or monoclonal antibody.

26. The cytotoxic agent of clause 25, wherein said cell binding agent is a monoclonal antibody.

27. The cytotoxic agent of clause 25 or 26, wherein said cell binding agent comprises at least one binding site of said antibody.

28. The cytotoxic agent of clause 1, wherein said cell binding agent is a hormone.

29. The cytotoxic agent of clause 28, wherein said hormone is melanocyte stimulating hormone (MSH) or an analogue thereof.

30. The cytotoxic agent of clause 28, wherein said hormone is thyroid stimulating hormone (TSH) or an analogue thereof.

31. The cytotoxic agent of clause 1, wherein said cell binding agent is a growth factor.

32. The cytotoxic agent of clause 31, wherein said growth factor is epidermal growth factor (EGF).

33. The cytotoxic agent of clause 31, wherein said growth factor is transforming growth factor alpha (TGF- α)

34. A therapeutic agent for killing selected cell populations comprising:

- (a) a cytotoxic amount of one or more maytansinoids linked to a cell binding agent, and
- (b) a pharmaceutically acceptable carrier, diluent or excipient.

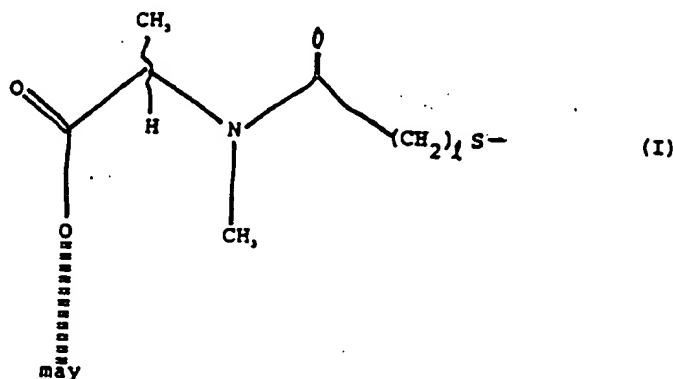
35. A method other than a method for treatment of the human or animal body by surgery or therapy or diagnostic methods practiced on the human or animal body for killing selected cell populations comprising contacting a cell population or tissue suspected of containing cells from said selected cell population with a cytotoxic amount of a cytotoxic agent comprising one or more maytansinoids linked to a cell binding agent.

36. An N-methyl-alanine-containing ester of maytansinol or an analogue of maytansinol, said N-methyl-alanine-containing ester comprising a linking group in the N-methyl-alanine moiety capable of chemically linking an N-methyl-alanine-containing maytansinoid ester to a chemical moiety via a disulfide band.

Claims

Claims for the following Contracting States : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

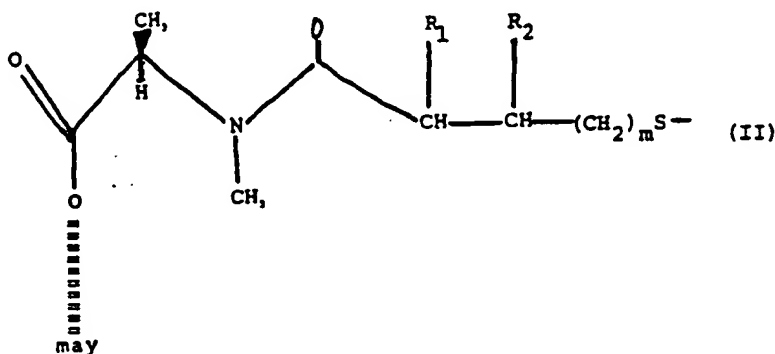
1. A cytotoxic agent comprising one or more maytansinoids chemically linked to a cell binding agent.
2. The cytotoxic agent of Claim 1, wherein the maytansinoid is a maytansinoid ester especially a C-3 maytansinoid.
3. The cytotoxic agent of Claim 2, wherein the maytansinoid ester is a N-methyl-alanine-containing maytansinoid ester.
4. The cytotoxic agent of Claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (I):



wherein:

l represents an integer of 1 to 10; and may represents a maytansinoid.

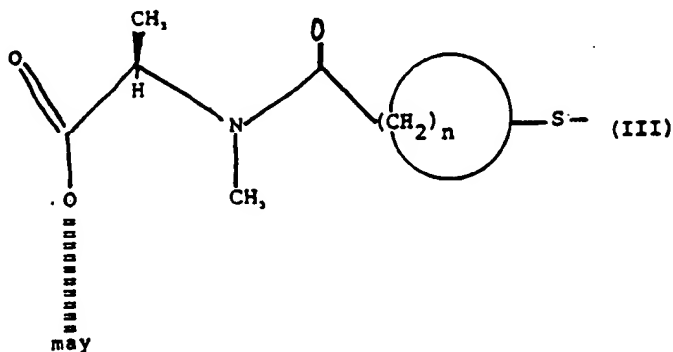
5. The cytotoxic agent of Claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (II):



wherein:

R_1 and R_2 , which may be the same or different, represents H,
 CH_3 or CH_2CH_3 ;
 m represents 0, 1, 2 or 3; and
 may represents a maytansinoid.

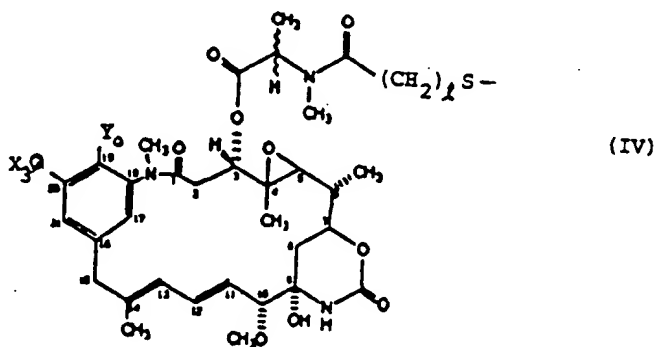
6. A cytotoxic agent of Claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (III);



wherein;

n represents an integer of 3 to 8; and may represents a maytansinoid.

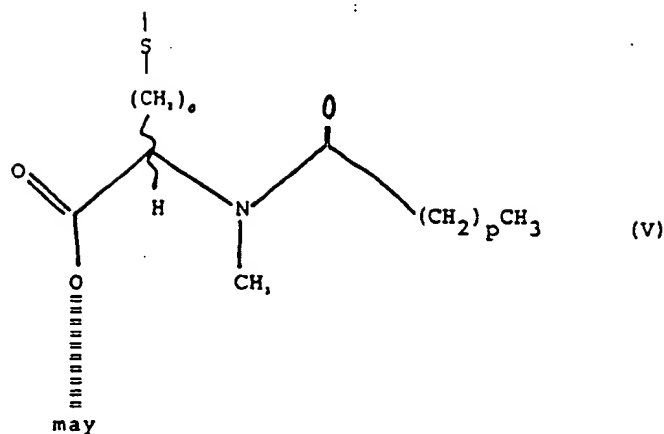
7. The cytotoxic agent of Claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is a C-3 ester of maytansinol.
8. The cytotoxic agent of Claim 7, wherein the maytansinoid ester is an ester of an analogue of maytansinol that has a modification at C-19 or C-20 or wherein the maytansinoid ester is an ester of dechloro maytansinol.
9. The cytotoxic agent of Claim 7, wherein the C-3 ester of maytansinol is represented by formula (IV):



wherein:

l represents 1, 2 or 3;
 Y_0 represents Cl or H; and
 X_3 represents H or CH_3 .

10. The cytotoxic agent of Claim 7 which comprises N-methyl-N-(3-methyldithiopropionyl)-L-alanine.
11. The cytotoxic agent of Claim 2, wherein the maytansinoid ester is an N-methyl-cysteine-containing maytansinoid ester preferably wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (V):



wherein:

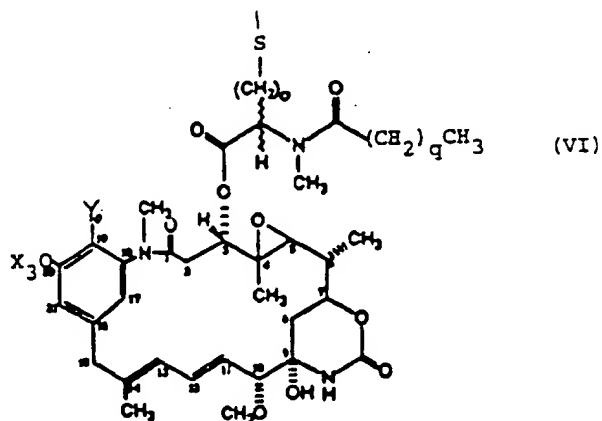
o represents 1, 2 or 3;

p represents 0 or an integer of 1 to 10; and

may represents a maytansinod.

12. The cytotoxic agent of Claim 11, wherein the N-methyl-cysteine-containing ester is a C-3 ester of maytansinol such as an ester of an analogue of maytansinol that has a modification at C-19 or C-10, or an ester of dechloro maytansinol.

13. The cytotoxic agent of Claim 11, wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (VI):



wherein:

o represents 1, 2 or 3;

q represents 0 or an integer of 1 to 10;

Y₀ represents Cl or H; and

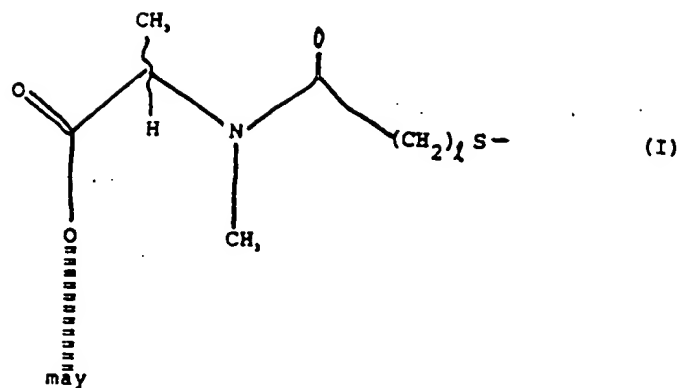
X₃ represents H or CH₃.

14. The cytotoxic agent of Claim 12, which comprises N-acetyl-N-methyl-methyldithiocysteine.

15. The cytotoxic agent of Claim 1, wherein the maytansinoid is chemically linked to the cell binding agent through a disulfide bond, a thioether bond, an acid labile chemical bond, a photo-labile chemical bond, or a chemical bond that can be cleaved by treatment with a peptidase or esterase.
- 5 16. The cytotoxic agent of Claim 1, wherein said cell binding agent is a peptide or a non-peptide.
17. The cytotoxic agent of Claim 1, wherein said cell binding agent is a polyclonal or monoclonal antibody.
- 10 18. The cytotoxic agent of Claim 17, wherein said cell binding agent comprises at least one binding site of said antibody.
19. The cytotoxic agent of Claim 1, wherein said cell binding agent is a hormone.
- 15 20. The cytotoxic agent of Claim 19, wherein said hormone is melanocyte stimulating hormone (MSH) or an analogue thereof or wherein said hormone is thyroid stimulating hormone (TSH) or an analogue thereof.
21. The cytotoxic agent of Claim 1, wherein said cell binding agent is a growth factor.
- 20 22. The cytotoxic agent of Claim 21, wherein said growth factor is epidermal growth factor (EGF), or wherein said growth factor is transforming growth factor alpha (TGF- α).
23. A therapeutic agent for killing selected cell populations comprising:
 - (a) a cytotoxic amount of one or more cytotoxic agents as claimed in any one of the preceding claims, and
 - (b) a pharmaceutically acceptable carrier, diluent or excipient.
- 25 24. A method other than a method for treatment of the human or animal body by surgery or therapy or diagnostic methods practiced on the human or animal body for killing selected cell populations comprising contacting a cell population or tissue suspected of containing cells from said and selected cell population with a cytotoxic amount of a cytotoxic agent as claimed in any one of claims 2 to 23.
- 30 25. An N-methyl-alanine-containing ester of maytansinol, said N-methyl-alanine-containing ester comprising a linking group in the N-methyl-alanine moiety capable of chemically linking an N-methyl-alanine-containing maytansinoid ester to a chemical moiety via a disulfide bond.
- 35 26. A cytotoxic agent as claimed in any one of Claims 1 to 22 for use as a medicament.
27. The use of a cytotoxic agent as claimed in any one of Claims 1 to 22 in the manufacture of a medicament for treating malignancy, for treating autoimmune diseases, for treating graft rejections, for treating viral infection or for treating parasite infection.
- 40

Claims for the following Contracting States : ES, GR

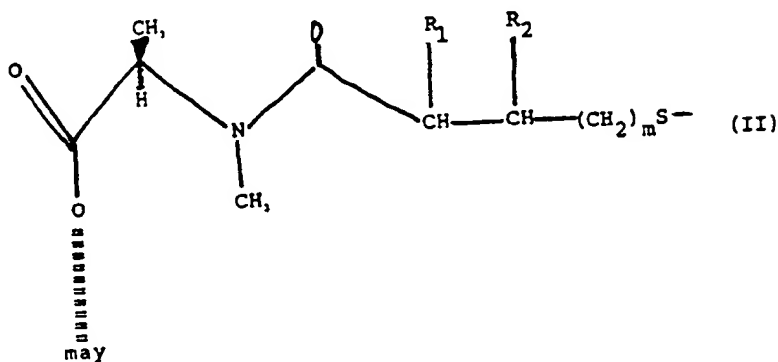
- 45 1. A method of manufacturing a cytotoxic agent comprising chemically linking one or more maytansinoids to a cell binding agent.
2. A method as claimed in claim 1, wherein the maytansinoid is a maytansinoid ester especially a C-3 maytansinoid ester.
- 50 3. A method as claimed in claim 2, wherein the maytansinoid ester is an N-methyl-alanine-containing maytansinoid ester.
4. A method as claimed in claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (I)
- 55



wherein:

l represents an integer of 1 to 10; and
may represents a maytansinoid.

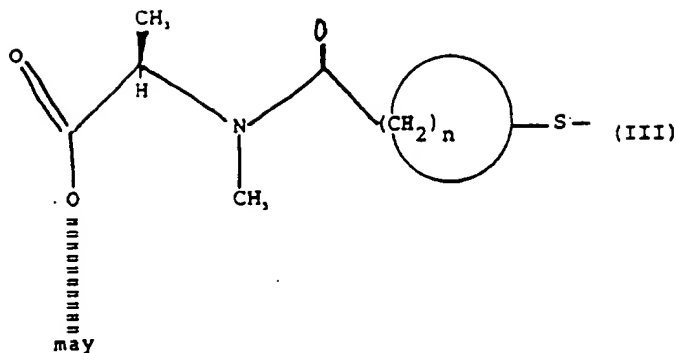
5. A method as claimed in claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (II)



wherein:

R₁ and R₂, which may be the same or different, represents H, CH₃ or CH₃CH₃;
m represents 0, 1, 2 or 3; and
may represents a maytansinoid.

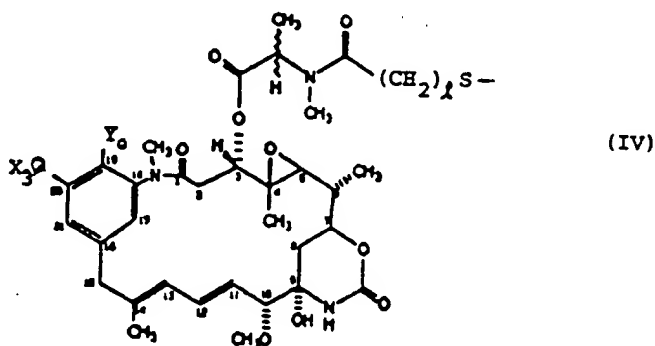
6. A method as claimed in claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is represented by formula (III):



wherein:

n represents an integer of 3 to 8; and
may represents a maytansinoid.

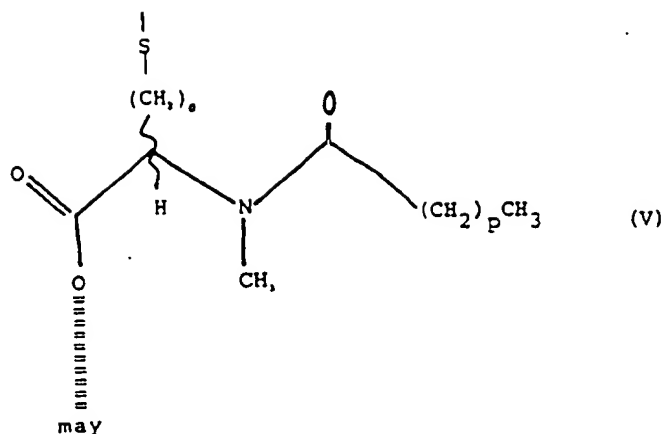
- 20
7. A method as claimed in claim 3, wherein the N-methyl-alanine-containing maytansinoid ester is a C-3 ester of maytansinol.
- 25
8. A method as claimed in claim 7, wherein the maytansinoid ester is an ester of an analogue of maytansinol that has a modification at C-19 or C-10 or wherein the maytansinoid ester is an ester of dechloro maytansinol.
9. A method as claimed in claim 7, wherein the C-3 ester of maytansinol is represented by formula (IV):



wherein

l represents 1, 2 or 3;
Y represents Cl or H; and
X₃ represents H or CH₃.

- 45
10. A method as claimed in claim 7, wherein the ester comprises N-methyl-N-(3-methyldithiopropionyl)-L-alanine.
- 50
11. A method as claimed in claim 2, wherein the maytansinoid ester is an N-methyl-cysteine-containing maytansinoid ester preferably wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (V):

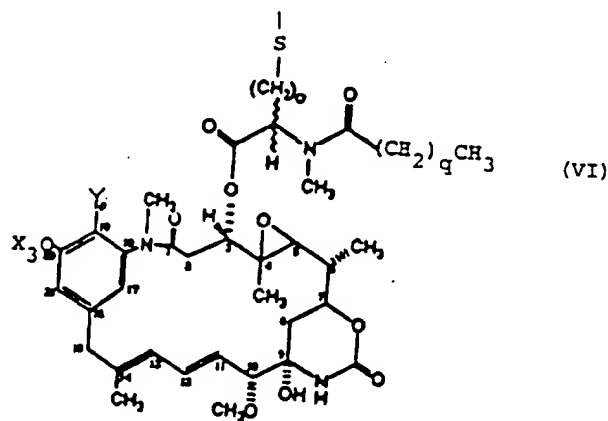


wherein:

o represents 1, 2 or 3;
p represents 0 or an integer of 1 to 10; and
may represents a maytansinod.

12. A method as claimed in claim 11, wherein the N-methyl-cysteine-containing ester is a C-3 ester of maytansinol such as an ester of an analogue of maytansinol that has a modification at C-19 or C-20, or an ester of dechloro maytansinol.

13. A method as claimed in claim 11, wherein the N-methyl-cysteine-containing maytansinoid ester is represented by formula (IV):



wherein:

o represents 1, 2 or 3;
q represents 0 or an integer of 1 to 10;
Y₀ represents Cl or H; and
X₃ represent H or CH₃.

14. A method as claimed in claim 12 wherein the ester comprises N-acetyl-N-methyl-methylidithiocysteine.

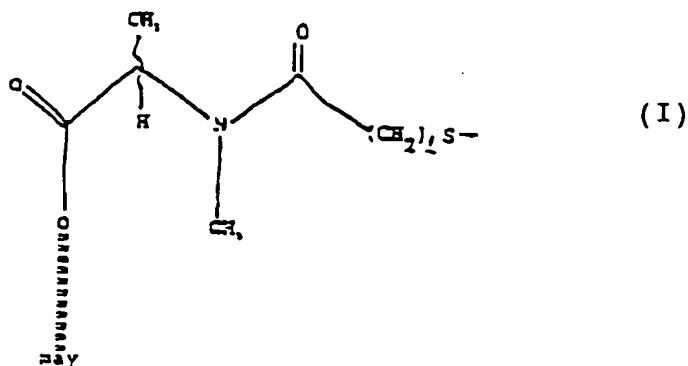
15. A method as claimed in claim 1 wherein the maytansinoid is chemically linked to the cell binding agent through a disulfide bond, a thioether bond, an acid-labile chemical bond, a photo-labile chemical bond, or a chemical bond that can be cleaved by treatment with a peptidase or esterase.

16. A method as claimed in claim 1, wherein said cell binding agent is a peptide or a non-peptide.
17. A method as claimed in claim 1, wherein said cell binding agent is a polyclonal or monoclonal antibody.
18. A method as claimed in claim 17, wherein said cell binding agent comprises at least one binding site of said antibody.
19. A method as claimed in claim 1, wherein said cell binding agent is a hormone.
20. A method as claimed in claim 1, wherein said hormone is melanocyte stimulating hormone (MSH) or an analogue thereof or where in said hormone is Thyroid Stimulating hormone (TSH) or an analogue thereof.

Patentansprüche

Patentansprüche für folgende Vertragsstaaten : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

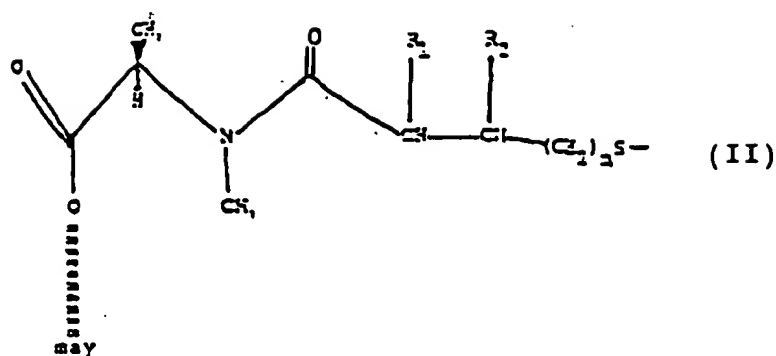
1. Cytotoxisches Mittel, das eines oder mehrere Maytansinoide umfaßt, welche chemisch mit einem Zellbindungsmittel verknüpft sind.
2. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Maytansinoid ein Maytansinoidester, insbesondere ein Maytansinoid-C3-Ester ist.
3. Cytotoxisches Mittel gemäß Anspruch 2, wobei der Maytansinoidester ein N-Methylalanin-haltiger Maytansinoidester ist.
4. Cytotoxisches Mittel gemäß Anspruch 3, wobei der N-Methylalanin-haltige Maytansinoidester durch die folgende Formel (I) dargestellt wird:



worin:

I für eine ganze Zahl von 1 bis 10 steht; und "may" für ein Maytansinoid steht.

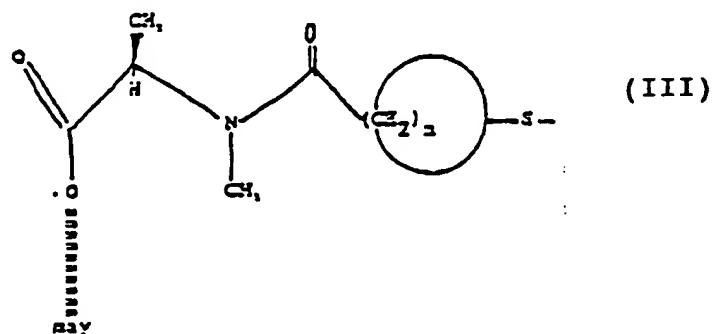
5. Cytotoxisches Mittel gemäß Anspruch 3, wobei der N-Methylalanin-haltige Maytansinoidester durch die folgende Formel (II) dargestellt wird:



15 worin:

20 R_1 und R_2 , welche gleich oder verschieden sein können, für H, CH_3 oder CH_2CH_3 stehen;
 m für 0, 1, 2 oder 3 steht; und
 may für ein Maytansinoid steht.

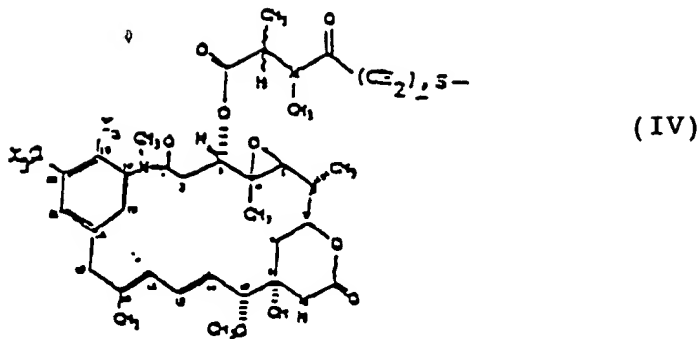
6. Cytotoxisches Mittel gemäß Anspruch 3, wobei der N-Methylalanin-haltige Maytansinoidester durch die folgende Formel (III) dargestellt wird:



35 worin:

40 n für eine ganze Zahl von 3 bis 8 steht; und may für ein Maytansinoid steht.

7. Cytotoxisches Mittel gemäß Anspruch 3, wobei der N-Methylalanin-haltige Maytansinoidester ein C3-Ester von Maytansinol ist.
8. Cytotoxisches Mittel gemäß Anspruch 7, wobei der Maytansinoidester ein Ester eines Analogens von Maytansinol ist, das eine Modifikation an C19 oder C20 aufweist, oder wobei der Maytansinoidester ein Ester von Dechloromaytansinol ist.
9. Cytotoxisches Mittel gemäß Anspruch 7, wobei der C3-Ester von Maytansinol durch die folgende Formel (IV) dargestellt wird:

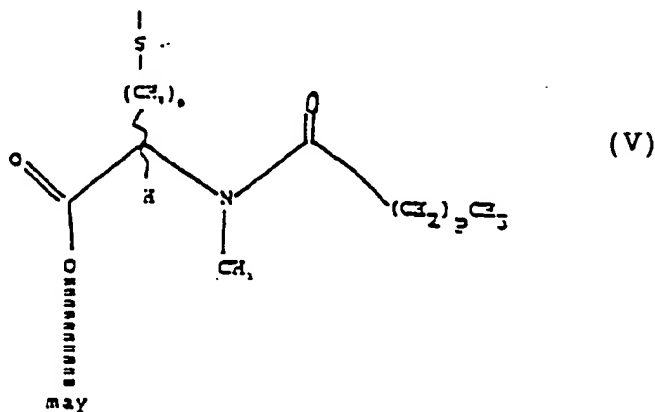


worin:

I für 1, 2 oder 3 steht;
 Y_0 für Cl oder H steht; und
 X_3 für H oder CH_3 steht.

10. Cytotoxisches Mittel gemäß Anspruch 7, welches N-Methyl-N-(3-methyldithiopropionyl)-L-Alanin enthält.

11. Cytotoxisches Mittel gemäß Anspruch 2, wobei der Maytansinoidester ein N-Methylcystein-haltiger Maytansinoidester ist, wobei vorzugsweise der N-Methylcystein-haltige Maytansinoidester durch die folgende Formel (V) dargestellt wird:

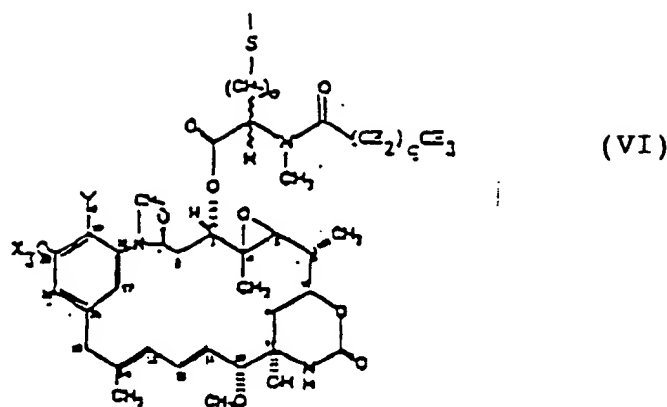


worin:

o für 1, 2 oder 3 steht;
p für 0 oder eine ganze Zahl von 1 bis 10 steht; und
may für ein Maytansinoid steht.

12. Cytotoxisches Mittel gemäß Anspruch 11, wobei der N-Methylcystein-haltige Ester ein C3-Ester von Maytansinol ist, so wie z.B. ein Ester eines Analogen von Maytansinol, welches eine Modifikation an C19 oder C20 aufweist, oder ein Ester von Dechloromaytansinol.

13. Cytotoxisches Mittel gemäß Anspruch 11, wobei der N-Methylcystein-haltige Maytansinoidester durch die folgende Formel (VI) dargestellt wird:



worin:

20 o für 1, 2 oder 3 steht;
 q für 0 oder eine ganze Zahl von 1 bis 10 steht;
 Y₀ für Cl oder H steht; und
 X₃ für H oder CH₃ steht.

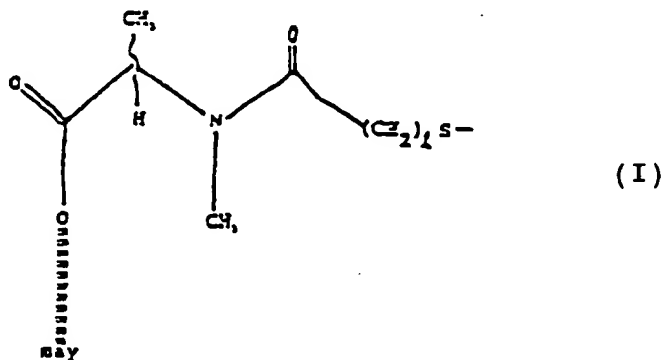
- 25 14. Cytotoxisches Mittel gemäß Anspruch 12, welches N-Acetyl-N-methyl-methyldithiocystein enthält.
15. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Maytansinoid chemisch über eine Disulfidbindung, eine Thioetherbindung, eine säurelabile chemische Bindung, eine photolabile chemische Bindung, oder eine chemische Bindung, die durch Behandlung mit einer Peptidase oder Esterase gespalten werden kann, mit dem Zellbindungsmittel verknüpft ist.
- 30 16. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Zellbindungsmittel ein Peptid oder ein nicht-Peptid ist.
17. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Zellbindungsmittel ein polyklonaler oder monoklonaler Antikörper ist.
- 35 18. Cytotoxisches Mittel gemäß Anspruch 17, wobei das Zellbindungsmittel wenigstens eine Bindungsstelle des Antikörpers umfaßt.
- 40 19. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Zellbindungsmittel ein Hormon ist.
20. Cytotoxisches Mittel gemäß Anspruch 19, wobei das Hormon Melanozyten-stimulierendes Hormon (MSH) oder ein Analoges davon ist, oder wobei das Hormon thyreotropes Hormon (TSH) oder ein Analoges davon ist.
- 45 21. Cytotoxisches Mittel gemäß Anspruch 1, wobei das Zellbindungsmittel ein Wachstumsfaktor ist.
22. Cytotoxisches Mittel gemäß Anspruch 21, wobei der Wachstumsfaktor epidermaler Wachstumsfaktor (EGF) ist oder wobei der Wachstumsfaktor transformierender Wachstumsfaktor alpha (TGF-α) ist.
- 50 23. Therapeutisches Mittel zum Töten von ausgewählten Zellpopulationen, umfassend:
- (a) eine cytotoxische Menge eines oder mehrerer cytotoxischer Mittel, wie sie in einem der vorhergehenden Ansprüche beansprucht sind, und
- (b) einen pharmazeutisch akzeptierbaren Träger, ein Verdünnungsmittel oder einen Arzneimittelträger.
- 55 24. Verfahren außer einem Verfahren zur Behandlung des menschlichen oder tierischen Körpers durch Chirurgie oder Therapie oder diagnostische Verfahren, welches am menschlichen oder tierischen Körper praktiziert wird, um ausgewählte Zellpopulationen zu töten, welches ein In-Kontakt-Bringen einer Zellpopulation oder eines Gewebes, von

welchem vermutet wird, daß es Zellen der ausgewählten Zellpopulation enthält, mit einer cytotoxischen Menge eines cytotoxischen Mittels, wie es in einem der Ansprüche 1 bis 22 beansprucht ist, umfaßt.

25. N-Methylalanin-haltiger Ester von Maytansinol, wobei der N-Methylalanin-haltiger Ester in der N-Methylalanin-Hälfte eine Verknüpfungsgruppe umfaßt, welche in der Lage ist, einen N-Methylalanin-haltigen Maytansinoidester über eine Disulfidbindung mit einer chemischen Hälfte chemisch zu verknüpfen.
26. Cytotoxisches Mittel gemäß einem der Ansprüche 1 bis 22 zur Verwendung als ein Medikament.
27. Verwendung eines cytotoxischen Mittels gemäß einem der Ansprüche 1 bis 22 bei der Herstellung eines Medikamentes zur Behandlung von bösartigen Tumoren, zur Behandlung von Autoimmunkrankheiten, zur Behandlung von Transplantatabstoßungen, zur Behandlung einer viralen Infektion oder zur Behandlung einer Infektion durch Parasiten.

Patentansprüche für folgende Vertragsstaaten : ES, GR

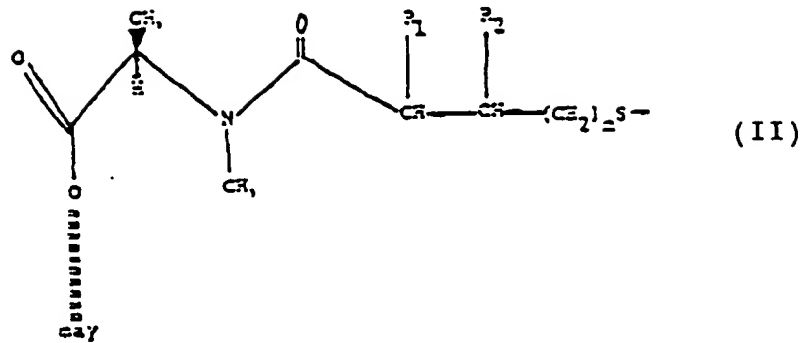
1. Verfahren zur Herstellung eines cytotoxischen Mittels, welches das chemische Verknüpfen eines oder mehrerer Maytansinoide mit einem Zellbindungsmittel umfaßt.
2. Verfahren gemäß Anspruch 1, wobei das Maytansinoid ein Maytansinoidester, insbesondere ein Maytansinoid-C3-Ester ist.
3. Verfahren gemäß Anspruch 2, wobei der Maytansinoidester ein N-Methylalanin-haltiger Maytansinoidester ist.
4. Verfahren gemäß Anspruch 3, wobei der N-Methylalaninhaltige Maytansinoidester durch die folgende Formel (I) dargestellt wird:



worin:

I für eine ganze Zahl von 1 bis 10 steht; und
may für ein Maytansinoid steht.

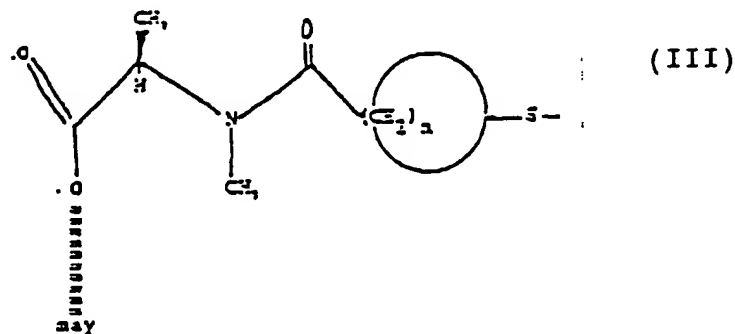
5. Verfahren gemäß Anspruch 3, wobei der N-Methylalaninhaltige Maytansinoidester durch die folgende Formel (II) dargestellt wird:



15
worin:

20
 R_1 und R_2 , welche gleich oder verschieden sein können, für H, CH_3 oder CH_2CH_3 stehen;
m für 0, 1, 2 oder 3 steht; und
may für ein Maytansinoid steht.

6. Verfahren gemäß Anspruch 3, wobei der N-Methylalaninhaltige Maytansinoidester durch die folgende Formel (III) dargestellt wird:

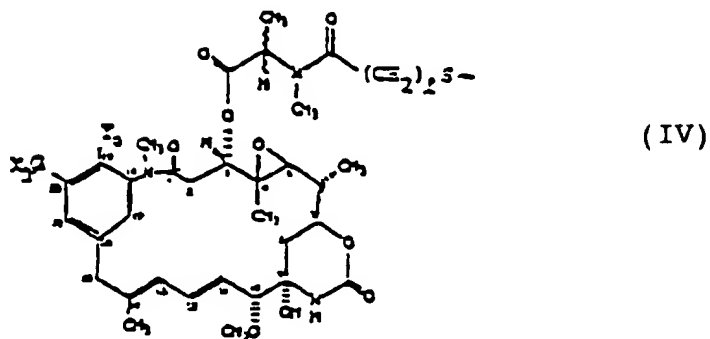


40
worin:

45
n für eine ganze Zahl von 3 bis 8 steht; und
may für ein Maytansinoid steht.

7. Verfahren gemäß Anspruch 3, wobei der N-Methylalaninhaltige Maytansinoidester ein C3-Ester von Maytansinol ist.
8. Verfahren gemäß Anspruch 7, wobei der Maytansinoidester ein Ester eines Analogens von Maytansinol ist, das eine Modifikation an C19 oder C20 aufweist, oder wobei der Maytansinoidester ein Ester von Dechloromaytansinol ist.

9. Verfahren gemäß Anspruch 7, wobei der C3-Ester von Maytansinol durch die folgende Formel (IV) dargestellt wird:

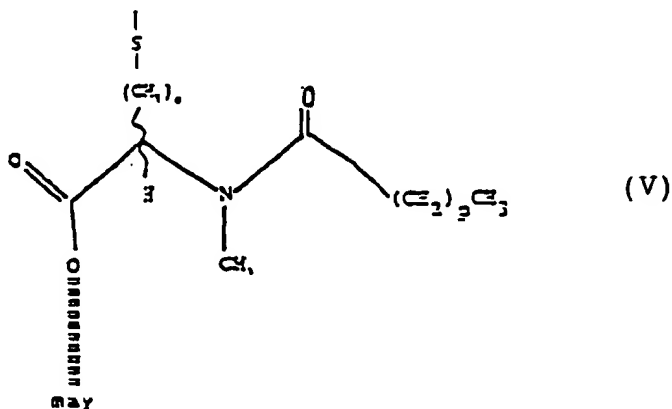


worin:

I für 1, 2 oder 3 steht;
 Y₀ für Cl oder H steht; und
 X₃ für H oder CH₃ steht.

10. Verfahren gemäß Anspruch 7, welches N-Methyl-N-(3-methyldithiopropionyl)-L-Alanin enthält.

11. Verfahren gemäß Anspruch 2, wobei der Maytansinoidester ein N-Methylcystein-haltiger Maytansinoidester ist, wobei vorzugsweise der N-Methylcystein-haltige Maytansinoidester durch die folgende Formel (V) dargestellt wird:

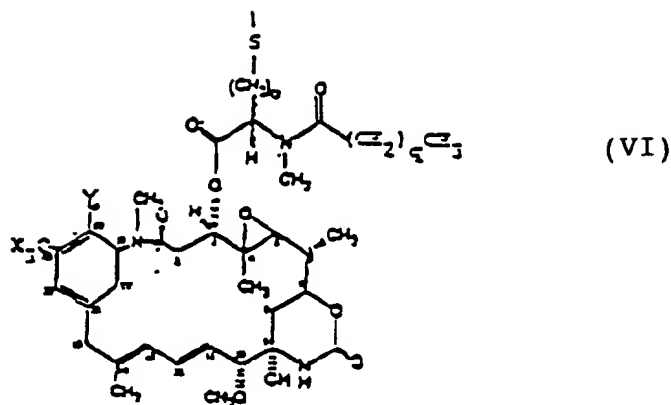


worin:

o für 1, 2 oder 3 steht;
 p für 0 oder eine ganze Zahl von 1 bis 10 steht; und
 may für ein Maytansinoid steht.

12. Verfahren gemäß Anspruch 11, wobei der N-Methylcysteininhaltige Ester ein C3-Ester von Maytansinol ist, so wie z.B. ein Ester eines Analogen von Maytansinol, welches eine Modifikation an C19 oder C20 aufweist, oder ein Ester von Dechloromaytansinol.

13. Verfahren gemäß Anspruch 11, wobei der N-Methylcysteininhaltige Maytansinoidester durch die folgende Formel (VI) dargestellt wird:



worin:

o für 1, 2 oder 3 steht;
 q für 0 oder eine ganze Zahl von 1 bis 10 steht;
 Y₀ für Cl oder H steht; und
 X₃ für H oder CH₃ steht.

14. Verfahren gemäß Anspruch 12, welches N-Acetyl-N-methylmethyldithiocystein enthält.

15. Verfahren gemäß Anspruch 1, wobei das Maytansinoid chemisch über eine Disulfidbindung, eine Thioetherbindung, eine säurelabile chemische Bindung, eine photolabile chemische Bindung, oder eine chemische Bindung, die durch Behandlung mit einer Peptidase oder Esterase gespalten werden kann, mit dem Zellbindungsmittel verknüpft ist.

16. Verfahren gemäß Anspruch 1, wobei das Zellbindungsmittel ein Peptid oder ein nicht-Peptid ist.

17. Verfahren gemäß Anspruch 1, wobei das Zellbindungsmittel ein polyklonaler oder monoklonaler Antikörper ist.

18. Verfahren gemäß Anspruch 17, wobei das Zellbindungsmittel wenigstens eine Bindungsstelle des Antikörpers umfaßt.

19. Verfahren gemäß Anspruch 1, wobei das Zellbindungsmittel ein Hormon ist.

20. Verfahren gemäß Anspruch 1, wobei das Hormon Melanozyten-stimulierendes Hormon (MSH) oder ein Analoges davon ist, oder wobei das Hormon thyreotropes Hormon (TSH) oder ein Analoges davon ist.

Revendications

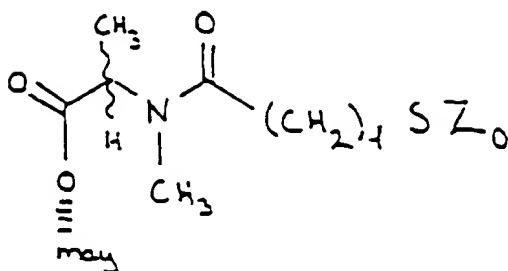
Revendications pour les Etats contractants suivants : AT, BE, CH, DE, DK, FR, GB, IT, LI, LU, NL, SE

1. Agent cytotoxique comportant au moins un Maytansinoïde chimiquement lié à un agent de liaison de cellule.

2. Agent cytotoxique selon la revendication 1, dans lequel le Maytansinoïde est un ester de Maytansinoïde, en particulier un C-3 ester de Maytansinoïde.

3. Agent cytotoxique selon la revendication 2, dans lequel l'ester de Maytansinoïde est un ester de Maytansinoïde contenant de la N-méthyl-alanine.

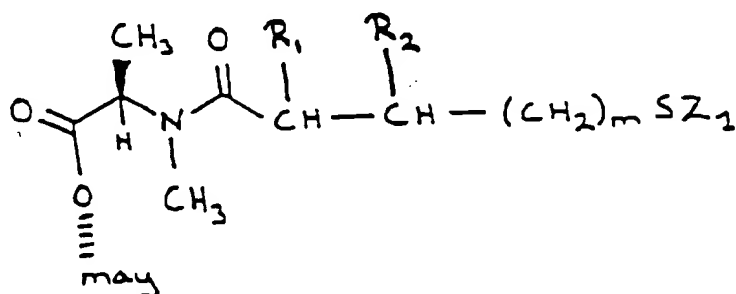
4. Agent cytotoxique selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant de la N-méthyl-alanine est représenté par la formule (I) :



dans laquelle :

I est un nombre entier de 1 à 10, et "may" représente un Maytansinoïde

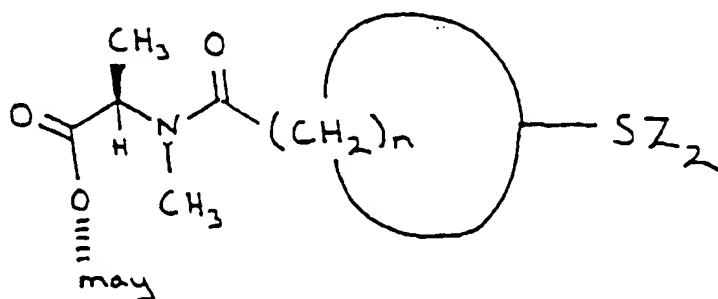
5. Agent cytotoxique selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant de la N-méthyl-alanine est représenté par le formule (II) :



dans laquelle

R₁ et R₂, qui peuvent être ou non différents représentent H, CH₃ ou CH₂CH₃;
m représente 0, 1, 2 ou 3 et;
"may" représente un Maytansinoïde.

6. Agent cytotoxique selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant la N-méthyl-alanine est représenté par la formule (III) :



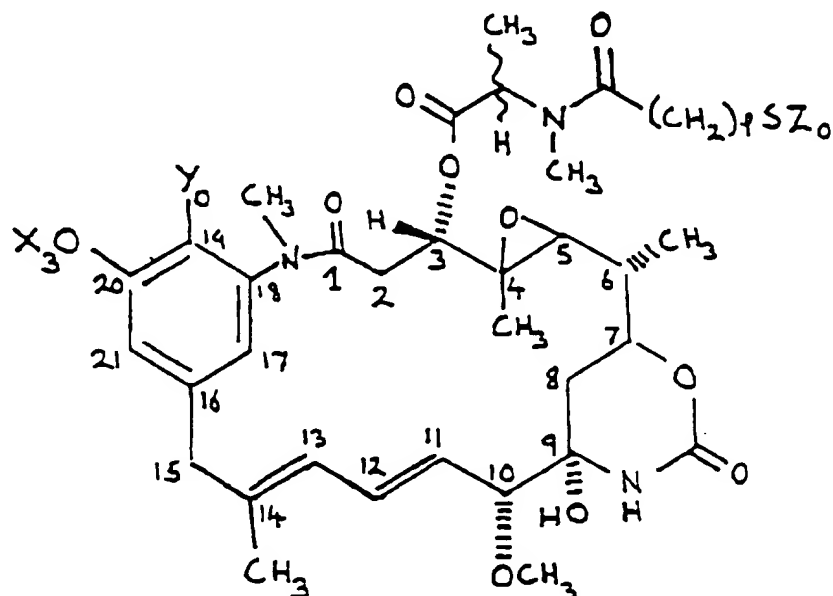
dans laquelle

n représente un nombre entier de 3 à 8 et may représente un Maytansinoïde.

7. Agent cytotoxique selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant du N-méthyl-alanine est un C-3 ester de maytansinol.

8. Agent cytotoxique selon la revendication 7, dans lequel l'ester de Maytansinoïde est un ester d'un analogue de maytansinol qui a une modification en C-19 ou C-20 ou dans lequel l'ester de Maytansinoïde est un ester de dechloro-maytansinol.

5 9. Agent cytotoxique selon la revendication 7, dans lequel le C-3 ester de maytansinol est représenté par la formule (IV) :

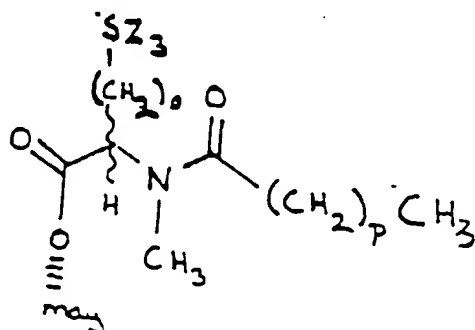


30 dans laquelle

I représente 1, 2 ou 3 ;
Y₀ représente Cl ou H; et
X₃ représente H ou CH₃.

35 10. Agent cytotoxique selon la revendication 7, qui comporte du N-méthyl-N-(3-méthylthiopropanoyl)-L-alanine.

40 11. Agent cytotoxique selon la revendication 2, dans lequel l'ester de Maytansinoïde est un ester de Maytansinoïde contenant du N-méthyl-cystéine, de préférence dans lequel l'ester de Maytansinoïde contenant le N-méthyl-cystéine est représenté par la formule (V) :

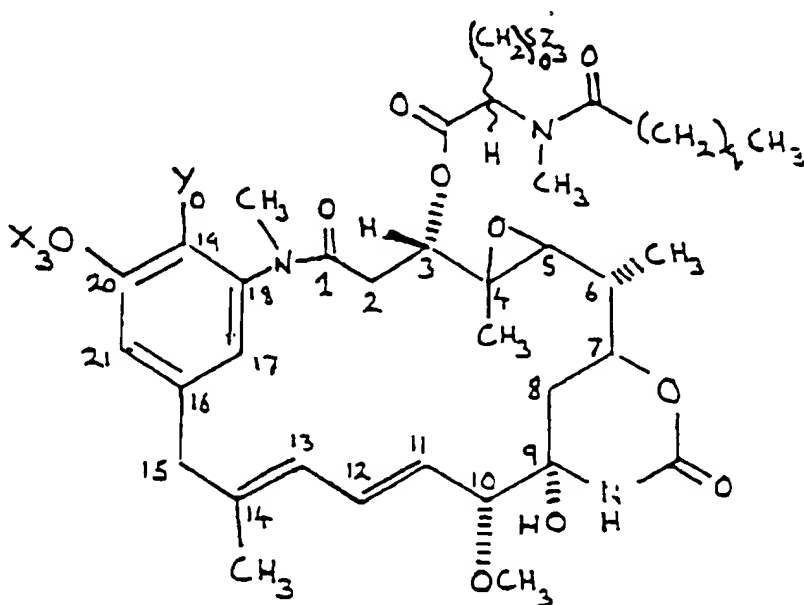


55 dans laquelle :

o représente 1, 2 ou 3 ;
p représente 0 ou un entier de 1 à 10 ; et

"may" représente un Maytansinoïde.

12. Agent cytotoxique selon la revendication 11 dans lequel l'ester contenant le N-méthyl-cystéine est un C3 ester de maytansinol tel qu'un ester d'un analogue du maytansinol, qui a une modification en C-19 ou en C-20, ou un ester de dechloro maytansinol.
13. Agent cytotoxique selon la revendication 11, dans lequel l'ester de Maytansinoïde contenant le N-méthyl-cystéine est représenté par la formule (VI) :



dans laquelle

o représente 1, 2 ou 3 ;
q représente 0 ou un entier de 1 à 10 ;
Y₀ représente Cl ou H; et
X₃ représente H ou CH₃

14. Agent cytotoxique selon la revendication 12, qui comporte du N-acétyl-N-méthyl-méthylidithiocystéine.
15. Agent cytotoxique selon la revendication 1, dans lequel le Maytansinoïde est chimiquement lié à l'agent de liaison de cellule par une liaison disulfure, une liaison thioether, une liaison chimique acide labile, une liaison chimique photo-labile, ou une liaison chimique qui peut être clivée par traitement avec un peptidase ou un esterase.
16. Agent cytotoxique selon la revendication 1, dans lequel ledit agent de liaison de cellule est un peptide ou un non-peptide.
17. Agent cytotoxique selon la revendication 1, dans lequel ledit agent de liaison de cellule est un anticorps polyclonal ou monoclal.
18. Agent cytotoxique selon la revendication 17, dans lequel ledit agent de liaison de cellule comporte au moins un site de liaison dudit anticorps.
19. Agent cytotoxique selon la revendication 1, dans lequel ledit agent de liaison de cellule est une hormone.
20. Agent cytotoxique selon la revendication 19, dans lequel ladite hormone est une hormone stimulant la melanocyte (MHS) ou une hormone analogue, ou dans laquelle ladite hormone est une hormone stimulant la thyroïde (TSH) ou une hormone analogue.

21. Agent cytotoxique selon la revendication 1, dans lequel ledit agent de liaison de cellule est un facteur de croissance.

22. Agent cytotoxique selon la revendication 21, dans lequel ledit facteur de croissance est un facteur de croissance épidermique (EGF), ou dans lequel ledit facteur de croissance est le facteur alpha de croissance transformant (TGF- α).

23. Agent thérapeutique pour tuer des populations de cellules sélectionnées comprenant :

- a) une quantité de cytotoxique d'au moins un agent cytotoxique tel que revendiqué dans une quelconque des revendications précédentes, et
- b) un support, diluant ou excipient pharmaceutiquement acceptable.

24. Procédé, autre qu'un procédé pour le traitement du corps humain ou animal par des procédés de chirurgie, de thérapie ou de diagnostic, pratiqués sur le corps humain ou animal pour tuer des populations de cellules sélectionnées, comportant la mise en contact d'une population de cellules ou d'un tissu soupçonné de contenir des cellules de ladite population de cellules sélectionnées, avec une quantité cytotoxique d'un Agent cytotoxique tel que revendiqué dans une quelconque des revendications 1 à 22.

25. Ester de maytansinol contenant du N-méthyl-alanine, ledit ester contenant du N-méthyl-alanine comportant un groupe de liaison dans la partie N-méthyl-alanine, capable de lier chimiquement un ester de Maytansinoïde contenant du N-méthyl-alanine à la partie chimique via une liaison disulfure.

26. Agent cytotoxique selon une quelconque des revendications 1 à 22 pour être utilisé comme un médicament.

27. Utilisation d'un agent cytotoxique selon une quelconque des revendications 1 à 22 pour la fabrication d'un médicament pour traiter la virulence, pour traiter les maladies autoimmunitaires, pour traiter les rejets de greffes, pour traiter les infections virales ou pour traiter les infections par parasites.

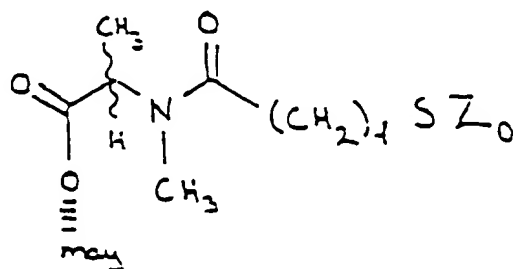
Revendications pour les Etats contractants suivants : ES, GR

1. Procédé de fabrication d'un agent cytotoxique comportant la liaison chimique d'au moins un Maytansinoïde à un agent de liaison de cellule.

2. Procédé selon la revendication 1, dans lequel le Maytansinoïde est un ester de Maytansinoïde, en particulier un C-3 ester de Maytansinoïde.

3. Procédé selon la revendication 2, dans lequel l'ester de Maytansinoïde est un ester de Maytansinoïde contenant de la N-méthyl-alanine.

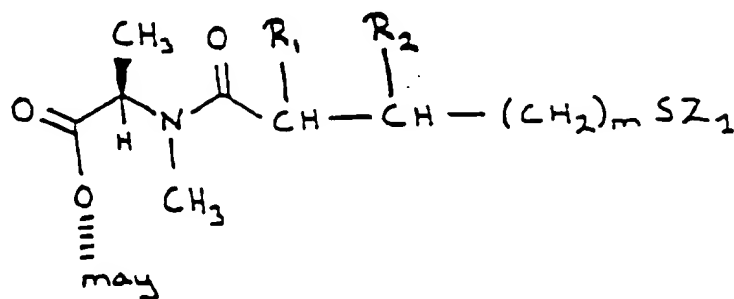
4. Procédé selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant de la N-méthyl-alanine est représenté par la formule (I) :



dans laquelle :

I est un nombre entier de 1 à 10, et "may" représente un Maytansinoïde

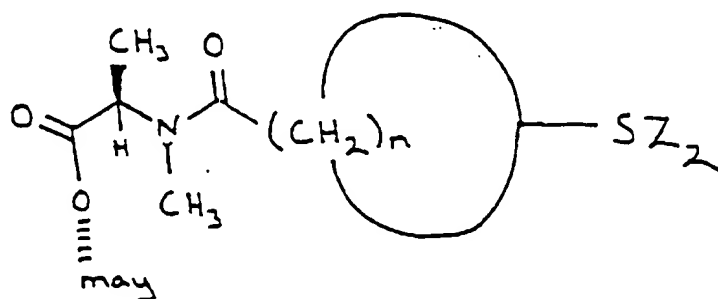
5. Procédé selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant de la N-méthyl-alanine est représenté par la formule (II) :



dans laquelle

R_1 et R_2 , qui peuvent être ou non différents représentent H, CH_3 ou CH_2CH_3 ;
 m représente 0, 1, 2 ou 3 et;
 "may" représente un Maytansinoïde.

6. Procédé selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant la N-méthyl-alanine est représenté par la formule (III) :

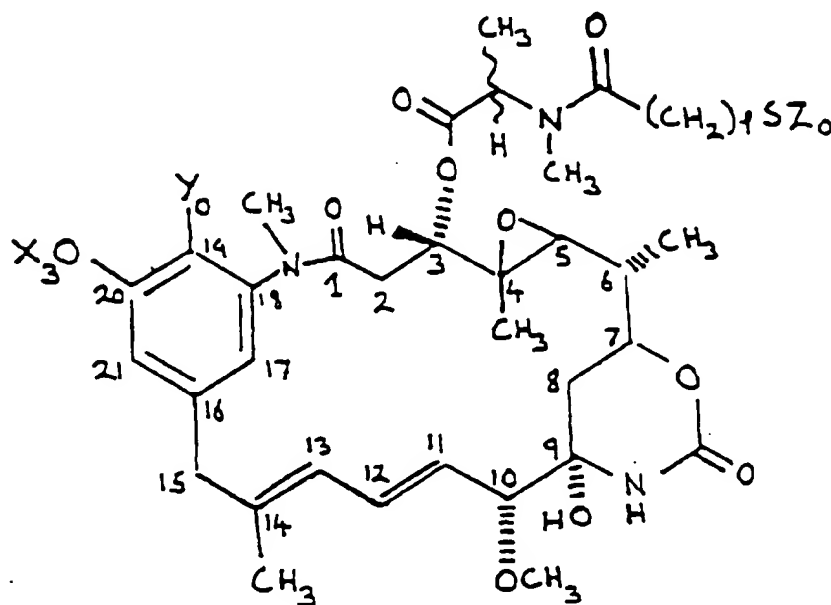


dans laquelle

n représente un nombre entier de 3 à 8 et may représente un Maytansinoïde.

7. Procédé selon la revendication 3, dans lequel l'ester de Maytansinoïde contenant du N-méthyl-alanine est un C-3 ester de maytansinol.
8. Procédé selon la revendication 7, dans lequel l'ester de Maytansinoïde est un ester d'un analogue de maytansinol qui a une modification en C-19 ou C-20 ou dans lequel l'ester de Maytansinoïde est un ester de dechloro-maytansinol.

9. Procédé selon la revendication 7, dans lequel le C-3 ester de maytansinol est représenté par la formule (IV) :

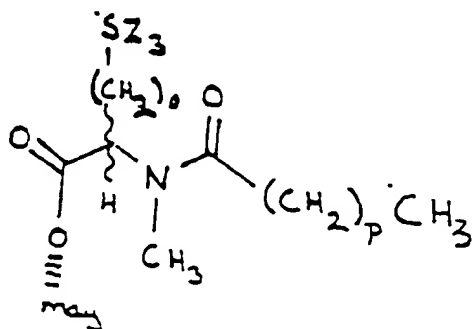


dans laquelle

l représente 1, 2 ou 3 ;
 Y_0 représente Cl ou H; et
 X_3 représente H ou CH_3 .

10. Procédé selon la revendication 7, qui comporte du N-méthyl-N-(3-méthylthiopropanoyl)-L-alanine.

11. Procédé selon la revendication 2, dans lequel l'ester de Maytansinoïde est un ester de Maytansinoïde contenant du N-méthyl-cystéine, de préférence dans lequel l'ester de Maytansinoïde contenant le N-méthyl-cystéine est représenté par la formule (V) :

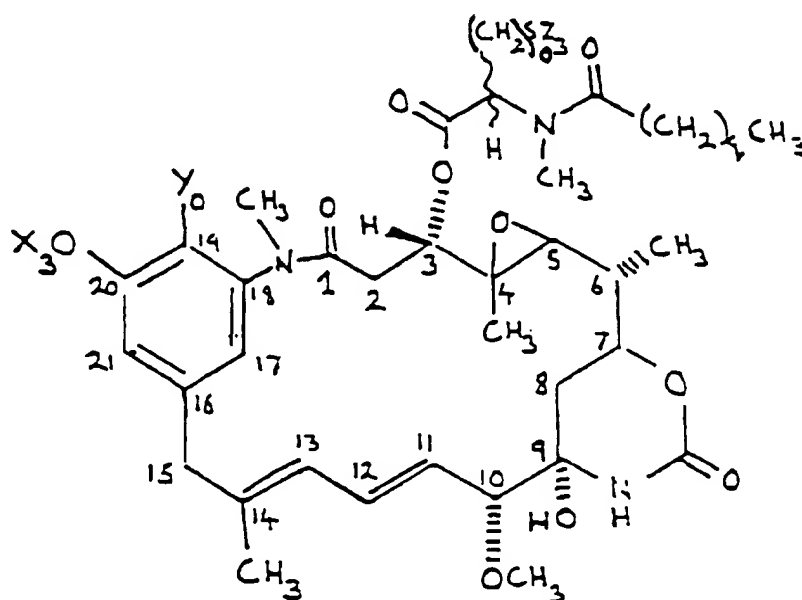


dans laquelle :

o représente 1, 2 ou 3 ;
p représente 0 ou un entier de 1 à 10 ; et
"may" représente un Maytansinoïde.

12. Procédé selon la revendication 11 dans lequel l'ester contenant le N-méthyl-cystéine est un C3 ester de maytansinol tel qu'un ester d'un analogue du maytansinol, qui a une modification en C-19 ou en C-20, ou un ester de dechloro maytansinol.

13. Procédé selon la revendication 11, dans lequel l'ester de Maytansinoïde contenant le N-méthyl-cystéine est représenté par la formule (VI) :



dans laquelle

o représente 1, 2 ou 3 ;
q représente 0 ou un entier de 1 à 10 ;
Y₀ représente Cl ou H ; et
X₃ représente H ou CH₃.

14. Procédé selon la revendication 12, qui comporte du N-acétyl-N-méthyl-méthylthiocystéine.
15. Procédé selon la revendication 1, dans lequel le Maytansinoïde est chimiquement lié à l'agent de liaison de cellule par une liaison disulfure, une liaison thioether, une liaison chimique acide labile, une liaison chimique photo-labile, ou une liaison chimique qui peut être clivée par traitement avec un peptidase ou un esterase.
16. Procédé selon la revendication 1, dans lequel ledit agent de liaison de cellule est un peptide ou un non-peptide.
17. Procédé selon la revendication 1, dans lequel ledit agent de liaison de cellule est un anticorps polyclonal ou monoclonal.
18. Procédé selon la revendication 17, dans lequel ledit agent de liaison de cellule comporte au moins un site de liaison dudit anticorps.
19. Procédé selon la revendication 1, dans lequel ledit agent de liaison de cellule est une hormone.
20. Procédé selon la revendication 19, dans lequel ladite hormone est une hormone stimulant la melanocyte (MHS) ou une hormone analogue, ou dans laquelle ladite hormone est une hormone stimulant la thyroïde (TSH) ou une hormone analogue.

FIG. 1 PRIOR ART

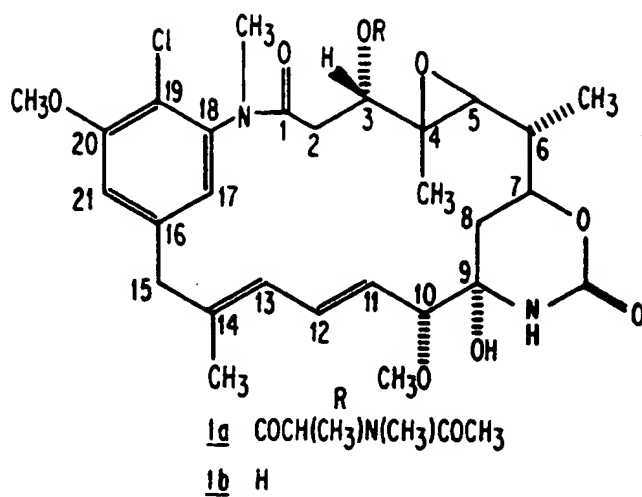


FIG. 2

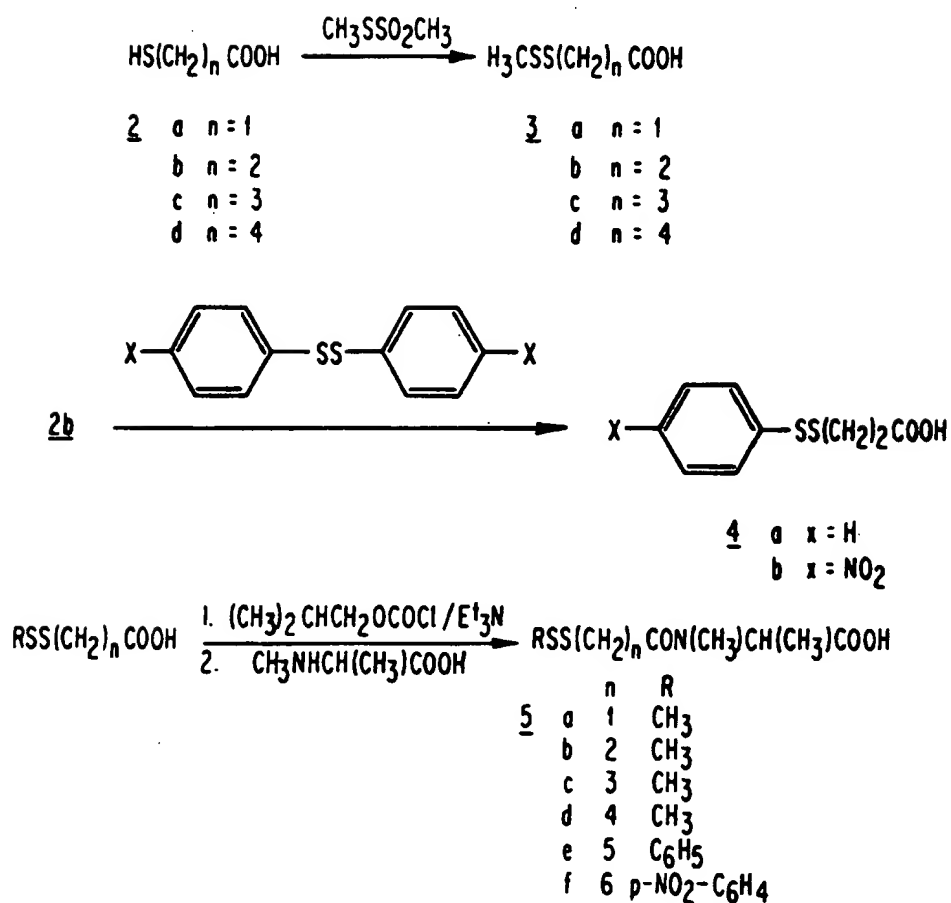


FIG. 3

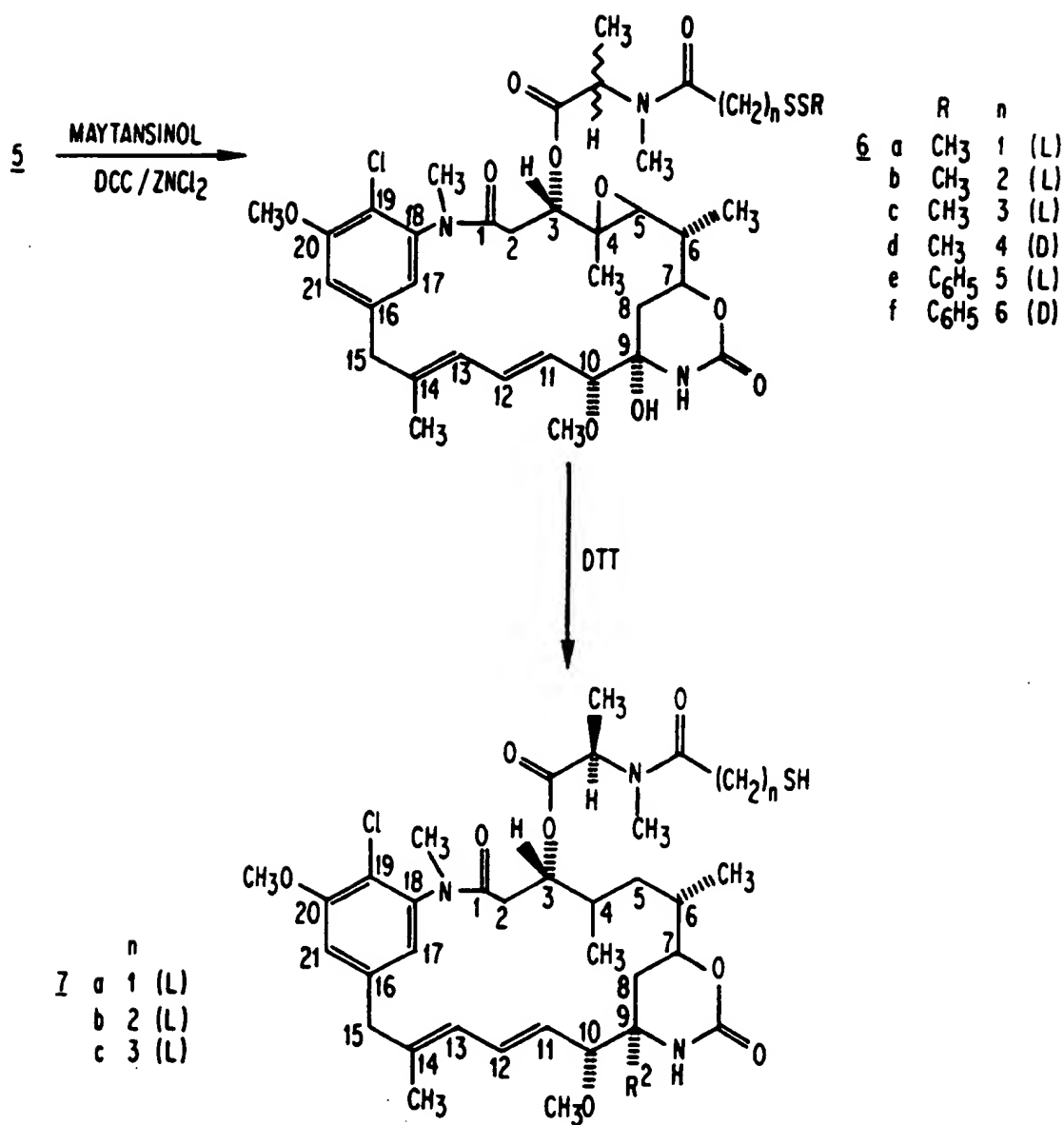


FIG. 4a

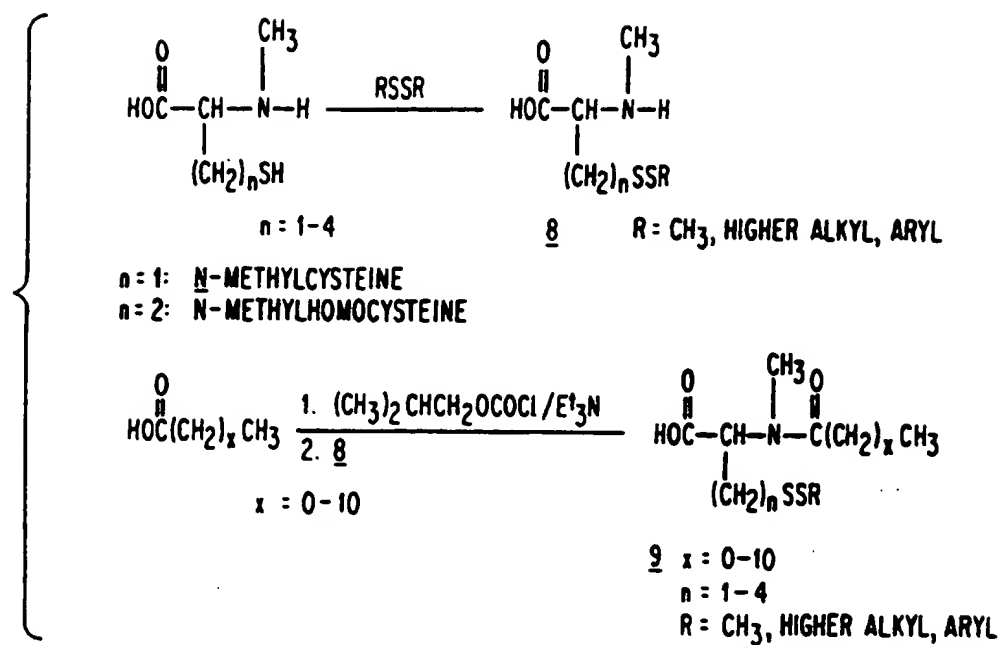


FIG. 4b

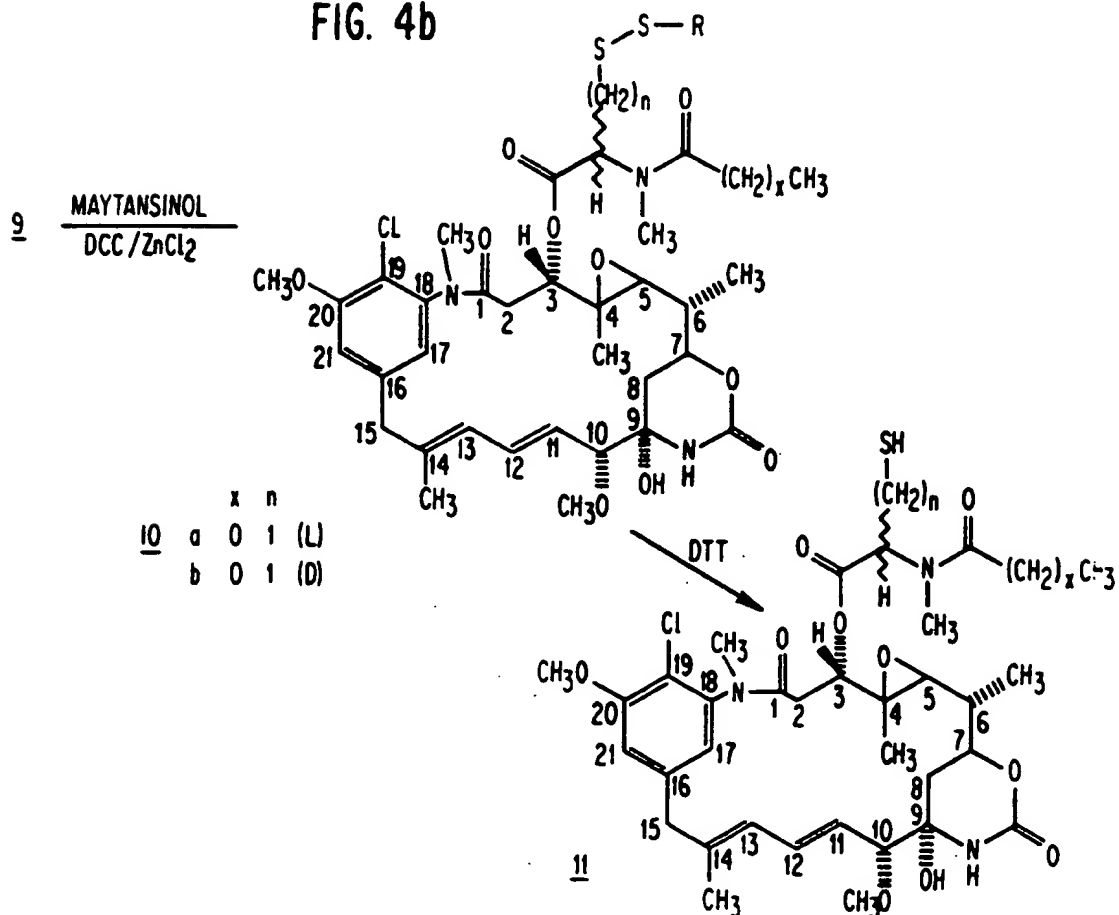


FIG. 5

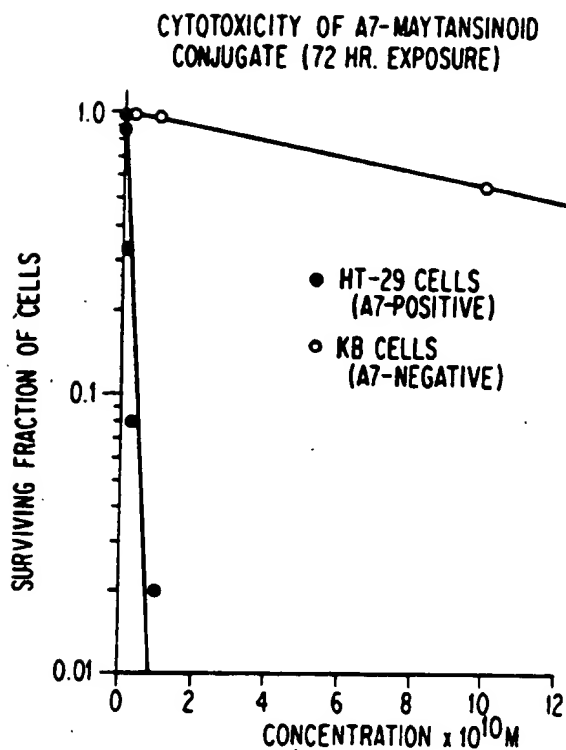


FIG. 6a

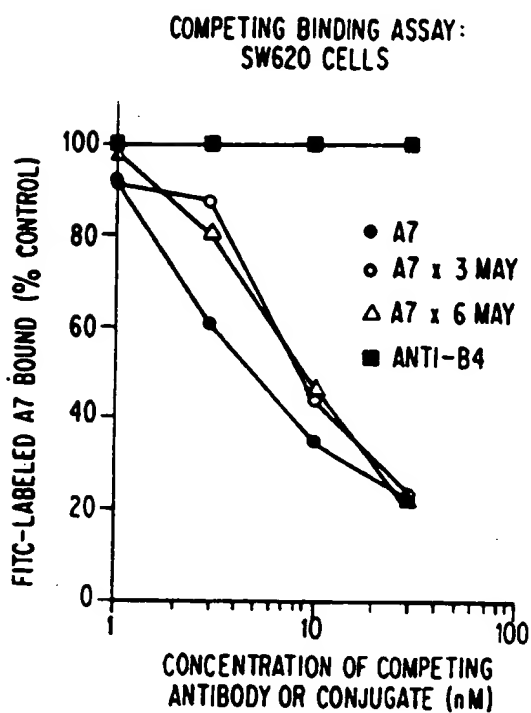
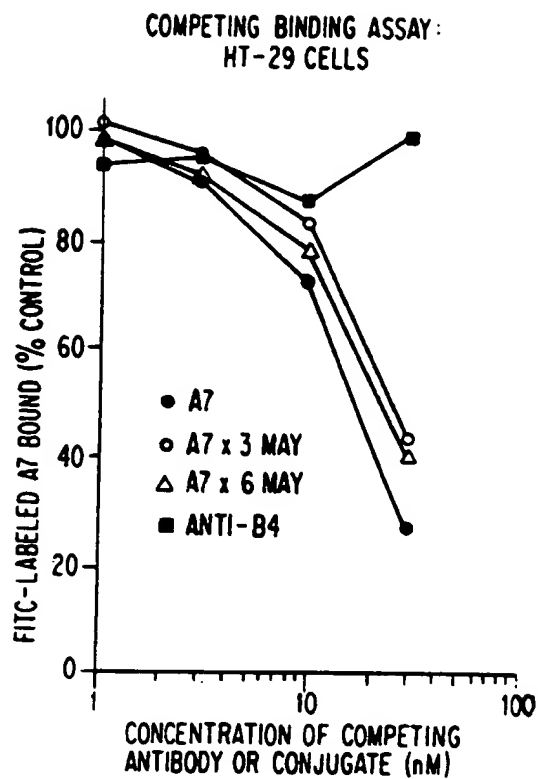


FIG. 6b

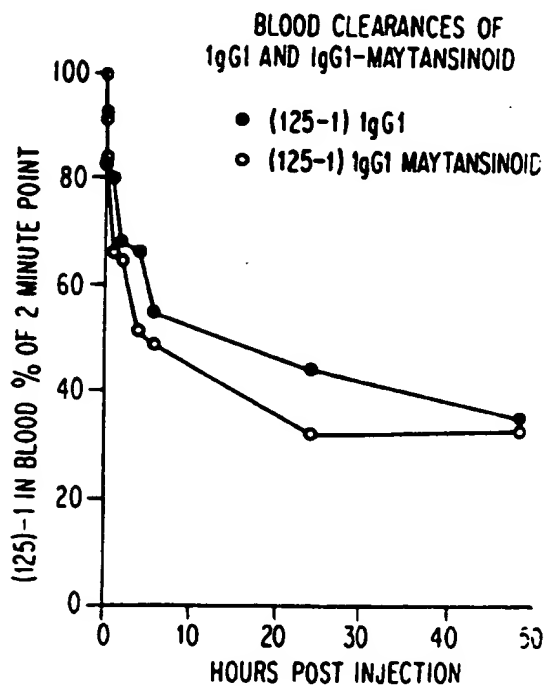


FIG. 7